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CANCER OF THE STOMACH IN THE LARGE TOWNS OF ENGLAND AND WALES, 1921–39.

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ANALYSIS has been made of the deaths from cancer of the stomach amongst residents in the 83 County Boroughs during the 19 years from 1921 to 1939 inclusive. Of these large towns other than London 42 are located in the northern region of England and 41 in the remainder of England and Wales, these groups being designated as " North " and " South " in this paper.

The deaths classified to cancer of stomach in 1921–30 and to cancer of stomach and duodenum in 1931–39 were aggregated by sex and age groups 0–, 15–, 25–, 35–, 45–, 55–, 65–, 75 and over. The total deaths in all County Boroughs, numbering 70,110, were divided by the census population at the centre of the period, 1931, and " expected ratios " of deaths to population obtained for each of the 16 sex-age groups. The census population of each town at each sex-age group was then multiplied by the corresponding " expected ratio," giving the " expected " deaths in that group in 1921–39. The actual deaths in a particular age group, or at all ages, divided by the expected deaths at the corresponding ages, or by the summation of them in all the age groups, and multiplied by 100, gave for each sex Comparative Mortality Ratios (C.M.R.) at each age group and at all ages. These are only approximate indices, since no correction has been made for irregular population trends in some of the towns ; but in no instance would the error from that cause exceed 5 per cent, and for only a few towns would it exceed 2 per cent.

In Table I the 83 towns are arranged in order of diminishing C.M.R. at all ages for cancer of the stomach in males. The values range from 130 to 55, those which are greater than 100 by at least twice the standard deviation being denoted by a + sign, and those less than 100 by twice the standard deviation are denoted by a − sign. The total deaths from cancer of the stomach are also shown.

The towns with C.M.R. in excess of 100 are divided into two approximately equal Groups (*a*, *b*) ; and those with C.M.R. not greater than 100 are again divided in the same way (*c*, *d*).

In each of the Groups *a*, *b*, *c*, *d*, the actual and expected deaths of all the towns were aggregated at ages 15–, 35–, 45–, 55–, 65–, 75 +, and the actual expressed

TABLE I.—*Cancer of Stomach in Towns.* 1921–39. *Males.*

County Borough.	C.M.R. 1921–39. All ages.	Significance.	By ages.	Number of deaths 1921–39.	Social class index 1931.	Age index 1931.	North or south.	Average hardness of water.	Ratio of deaths 1931–39 per 1000 population in 1931. Peptic ulcer. 45–.	Peptic ulcer. 65+.	Other digestive.* 45–.	Other digestive.* 65+.
Salford	130	+		759	42·4	18	N.	1·5	5·1	4·7	4·8	21·2
Birkenhead	129	+		514	41·8	19	N.	10	4·1	5·6	4·3	19·6
Rochdale	128	+		356	39·2	22	N.	3·4	4·9	7·1	7·9	28·4
Liverpool	126	+		2691	43·4	18	N.	8	4·8	5·0	5·3	16·7
Bootle	125	+		239	50·4	18	N.	8	4·9	9·0	3·4	19·2
West Hartlepool	125	+		240	45·4	20	N.	34	3·4	4·9	5·6	15·7
Oldham	124	+	Group *a*.	518	37·6	20	N.	4	4·0	3·8	6·4	19·8
Swansea	123	+		574	39·0	19	S.	2	4·6	4·6	5·6	18·2
Sunderland	122	+	15– : 125	596	41·2	20	N.	25	3·7	4·2	5·9	14·0
Stockport	120	+	35– : 129	454	34·3	21	N.	4	3·9	3·9	4·3	19·3
Stoke-on-Treat	120	+	45– : 126	821	40·4	18	S.	10·5	3·2	5·4	6·5	22·8
Manchester	119	+	55– : 122	2433	35·2	19	N.	2	4·2	5·6	5·0	20·3
Chester	119	+	65– : 119	140	35·2	20	N.	7	5·6	5·3	8·0	21·9
Bury	119	+	75+: 115	216	38·8	22	N.	5·2	4·7	4·0	7·0	24·2
Gateshead	117	+		387	43·1	20	N.	15·5	3·6	3·5	5·7	21·9
West Ham	117	+		887	48·3	18	S.	7	4·7	6·9	4·2	19·6
Carlisle	117	+		193	37·7	21	N.	7	5·2	8·1	5·6	23·7
Preston	117	+		381	37·3	20	N.	3·5	4·2	3·7	5·7	26·1
Kingston-on-Hull	115	+		990	47·0	19	N.	20	4·6	6·3	7·1	24·7
South Shields	115	+		351	43·1	20	N.	25	3·6	3·0	6·2	22·6
Wigan	113	..		260	42·0	19	N.	7·6	4·0	3·4	6·0	20·4
Grimsby	113	+		317	52·0	20	S.	23	4·5	3·2	6·1	21·9
Blackburn	112	+		431	32·2	20	N.	1·6	4·4	5·0	6·0	20·3
Dewsbury	111	..		186	39·7	24	N.	3·2	4·2	4·3	5·2	22·3
Newcastle	110	+		865	38·6	20	N.	14·5	4·5	5·2	6·0	21·8
Wallasey	109	..	Group *b*.	318	24·9	22	N.	9·6	5·1	8·2	5·8	23·2
St. Helens	109	..		301	48·0	18	N.	15	2·7	2·1	5·5	22·3
Newport	108	..	15– : 90	281	41·3	20	S.	8	4·5	5·4	7·1	23·5
Huddersfield	107	..	35– : 103	381	34·3	25	N.	4·2	4·6	6·8	6·1	25·2
Middlesbrough	106	..	45– : 104	385	49·4	18	N.	4·7	3·0	2·8	5·9	17·0
Southport	105	..	55– : 105	279	21·7	25	N.	19	5·2	7·1	8·1	22·2
Sheffield	104	..	65– : 108	1516	38·2	20	N.	2·3	4·1	4·2	5·3	19·7
East Ham	103	..	75+: 108	421	31·1	19	S.	7	4·3	6·4	5·1	24·1
Dudley	102	..		154	38·2	17	S.	12	5·3	3·5	4·6	21·9
Barrow	102	..		215	38·5	19	N.	2·5	4·6	2·6	4·6	17·9
Halifax	102	..		329	36·7	24	N.	6·5	4·0	1·9	6·9	21·6
Cardiff	102	..		672	35·8	21	S.	3·2	4·7	4·9	6·3	19·3
Warrington	102	..		213	46·5	18	N.	18	3·6	3·7	4·2	29·3
Southampton	101	..		559	33·9	22	S.	7·5	4·5	6·8	5·3	18·7

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TABLE II.—*Cancer of Stomach in Towns.* 1921–1939. *Females.*

County Borough.	C.M.R. 1921–39. All ages.	Significance.	By ages.	Number of deaths 1921–39.	Social class index 1931.	Age index 1931.	North or south.	Average hardness of water.	Ratio of deaths 1931–39 per 1000 population in 1931. Peptic ulcer. 45–.	Peptic ulcer. 65+.	Other digestive.* 45–.	Other digestive.* 65+.
Bootle	142	+		206	50·4	18	N.	8	0·7	2·7	4·5	18·3
Oldham	137	+		456	37·6	21	N.	4	0·7	1·8	6·3	25·2
Rochdale	136	+		323	39·2	23	N.	3·4	1·1	1·1	8·1	29·9
Swansea	135	+		452	39·0	19	S.	2	0·7	3·0	5·0	20·6
St. Helens	134	+		238	48·0	18	N.	15	0·8	2·7	8·7	25·2
Sunderland	134	+		498	41·2	20	N.	25	0·7	0·8	5·2	18·1
Birkenhead	132	+	Group *a*.	432	41·8	21	N.	10	1·1	2·1	6·0	23·8
South Shields	132	+		297	43·1	20	N.	25	0·4	0·9	5·2	19·9
Carlisle	129	+	15– : 129	180	37·7	21	N.	7	1·1	1·7	6·2	18·0
Liverpool	126	+	35– : 121	2199	43·4	19	N.	8	1·2	1·9	4·8	19·5
Salford	126	+	45– : 131	593	42·4	19	N.	1·5	1·2	2·1	5·7	24·5
Stoke-on-Trent	126	+	55– : 131	645	40·4	18	S.	10·5	1·2	1·9	5·5	22·4
Dewsbury	126	+	65– : 128	178	39·7	23	N.	3·2	0·7	1·3	6·6	20·7
Stockport	126	+	75+ : 120	413	34·3	23	N.	4	1·0	2·7	6·8	19·9
Barnsley	123	+		158	40·8	19	N.	2·5	1·0	3·1	5·7	30·2
Gateshead	122	+		295	43·1	20	N.	15·5	0·9	1·1	5·8	19·8
Merthyr Tydfil	122	+		166	42·5	20	S.	4·1	0·8	0·5	5·9	26·8
Newcastle	122	+		709	38·6	20	N.	14·5	0·9	2·0	7·2	20·7
Barrow	122	+		168	38·5	21	N.	2·5	0·7	1·0	4·2	16·2
Burnley	119	+		292	30·7	22	N.	3	1·4	1·6	7·2	31·5
Manchester	117	+		1990	35·2	20	N.	2	1·0	2·0	4·8	21·0
Wigan	116	+		192	42·0	19	N.	7·6	0·8	0·4	6·2	24·4
Blackburn	114	+		383	32·2	23	N.	1·6	1·0	2·2	6·5	20·3
Preston	114	+		329	37·3	22	N.	3·5	1·3	2·0	6·2	22·4
Chester	112	..		113	35·2	22	N.	7	1·5	3·5	6·4	20·5
Middlesbrough	112	..		273	49·4	18	N.	4·7	1·3	0·5	6·6	20·3
Newport	111	..		210	41·3	20	S.	8	0·9	1·3	5·1	24·2
East Ham	111	..	Group *b*.	356	31·1	20	S.	7	0·6	5·1	4·4	18·8
West Ham	111	+	15– : 103	650	48·3	19	S.	7	1·0	2·3	4·8	17·3
Huddersfield	110	..	35– : 115	342	34·3	23	N.	4·2	1·2	2·4	6·0	23·4
Halifax	109	..	45– : 104	319	36·7	25	N.	6·5	1·2	1·8	4·5	17·3
Bradford	108	+	55– : 109	899	36·1	24	N.	2·8	0·9	1·9	5·7	21·7
Kingston-on-Hull	108	+	65– : 113	705	47·0	20	N.	20	1·0	2·8	5·4	19·1
Darlington	107	..	75+ : 111	178	35·7	21	N.	6·7	0·9	1·5	5·7	27·2
West Hartlepool	107	..		146	45·4	20	N.	34	1·3	..	5·7	17·1
Wallasey	105	..		292	24·9	24	N.	9·6	1·1	3·0	4·6	21·3
Wakefield	105	..		138	36·9	21	N.	5·3	1·1	2·4	6·6	23·9
Warrington	104	..		148	46·5	18	N.	18	1·1	1·9	6·2	21·7
Cardiff	104	..		506	35·8	20	S.	3·2	1·2	2·0	4·4	18·9
Blackpool	102	..		361	25·4	21	N.	3·3	1·3	1·4	8·1	27·8
Southport	102	..		310	21·7	29	N.	19	1·0	1·4	5·3	22·9

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as a percentage of the expected deaths, giving a series of group C.M.R.'s, indicating how the relative excess or deficiency of mortality characterizing the towns within the group changed according to age.

Table I shows also the following characteristics of the towns :

Social Class Index (S.C.I.) : Percentage of all employed males in 1931 who were in unskilled or partly skilled occupations (i.e. social classes IV and V).

Age Index (A.I.) : Percentage of males (or females) aged 15 and over who were aged 55 and over in 1931.

Degrees of total hardness of water supply (H) : Average value estimated for the period 1910–1931, expressed as parts per 70,000.

North or South : Whether situated in the Northern Region of England or elsewhere in England and Wales.

Peptic ulcer indices : Ratios of deaths from gastric and duodenal ulcer in 1931–39 to population in 1931 at ages 45–65 and over 65.

Other digestive indices : Similar ratios for deaths from cancer of the liver and causes included in the international groups for diseases of the digestive system except appendicitis and peptic ulcer.

Table II gives the same information for females.

In Table I 23 of the towns had C.M.R.'s exceeding 100 by twice their standard deviation or more, and 30 had C.M.R.'s less than 100 by that amount. No more than 4 out of the 83 would be expected to give values differing from 100 by as much as this on account of random variations due to a small number of deaths, so it is evident that the deviations for 49 of the towns cannot be so accounted for.

In Table II the range of C.M.R. was from 142 to 61, with 27 towns showing significant excess over 100 and 27 a significant deficiency, so the deviations for 50 of the towns cannot be accounted for by random variation.

Group (*a*) for males comprises 20 towns with C.M.R. exceeding 114, and for females 20 towns with C.M.R. exceeding 118, all the values being significantly greater than 100.

For each sex 17 of the 20 towns in this group were in the North of England.

Group (*d*) for males comprises 18 towns with C.M.R. below 80, all being significantly below 100, and of these only 3 were in the North. For females it comprises 20 towns with C.M.R. below 83, all significantly below 100, and of these only one was in the North.

Because of the strong tendency for towns in the North to have higher mortality from cancer of the stomach, correlation co-efficients between the C.M.R. and the Social Class Index on the one hand and the Age Index on the other have been calculated separately for the 42 Northern towns and 41 other towns, and also for the 83 together. The values given in Table III show that cancer of stomach mortality is positively correlated with the proportion of unskilled workers in the population, more so in south than north ; and in England and Wales as a whole the coefficients are about $+\cdot5$ for each sex. There is, however, a strong negative correlation between the social class index and age index, meaning that towns with larger proportions of unskilled labour tend to have smaller proportions of their adult populations with ages over 55. When the age index is kept constant cancer of stomach is still positively correlated with social class index to the extent of $+\cdot3$ to $+\cdot4$, and this agrees with a similar result for the Metropolitan Boroughs of London in 1921–30 (Registrar-General, 1948). It was to be expected from the pronounced gradient of cancer of stomach mortality upon

social class of the whole population in 1930–32, as demonstrated in the Occupational Mortality Supplement (Registrar-General, 1931).

It is now seen that there is also a small negative correlation between cancer of stomach and the proportion of people of the same sex alive at ages 55 and over, and that this does not disappear when the social class index is made constant. This is important, because it shows that the great variation in cancer of stomach rates in the towns is not due to mere differences in the correctness of certification of this cause of death by doctors. It has been suggested that the notable differences between cancer of stomach rates recorded in Britain, Denmark and Switzerland may be due to excessive statement of this as cause of death in parts of those countries. If that were so it would be expected that the over-statement would be greater amongst old than amongst young people, since the care devoted to diagnosis and correct statement of cause of death inevitably diminishes after about age 55. Consequently, if the great differences in standardized death rates in different towns, and in social grades, were due to excessive statement of cancer of stomach in certain of them, those with high rates would tend to have larger proportions of old people. But in fact the reverse is the case both for the towns and for the social classes.

This can be shown in another way, by comparing the group C.M.R.'s at different ages in Tables I and II. The differences and ratios between the C.M.R. indices for the towns of very high and very low mortality at successive ages are shown in Table IV for each sex. For males the contrast is greatest at ages between 35 and 55, when the death rates in the aggregate of 20 towns forming Group (*a*) were just double those in the 18 towns forming Group (*d*); and after 55 the relative excess diminishes to only 35 per cent at ages 75 and over. For females the excess in Group (*a*) compared with Group (*d*) was around 90 per cent up to age 65, and then diminished to 45 per cent at 75 and over.

A pronounced decrease in the dispersion of death rates as age advances after 45 in males and after 55 in females is to be seen in Table V, where rates for cancer of the stomach in the different social classes of the population of England and Wales in 1930–32 are compared in the same way. For males the excess mortality in Class V, unskilled labourers, compared with that in Classes I–II, professional and higher graded occupations, was as great as 86 per cent at 35–45, and diminished progressively to only 8 per cent at 70 and over. For the wives of men in Class V and single women in unskilled occupations the excess was 80 per cent at 45–55, and fell to 37 per cent at 70 and over.

These relations with age are the reverse of what would be expected if the large differences in causes of stomach mortality between various large towns and between social classes arose from differing accuracy of death certification. The wide differences in the various parts of London are also incapable of such an explanation (Registrar-General, 1947). It will be seen from Table 13 of that publication that the dispersion of rates according to a housing density grouping were much smaller at ages over 65 than at 45–65.

More convincing evidence even than this is furnished by the correlations between cancer of stomach death rates and those for other digestive diseases in Table VI. If the differences between cancer rates in Groups (*a*) and (*d*) of Table IV were due to more complete diagnosis, or excessive diagnosis, of stomach cancer in (*a*) than (*d*), there must have been corresponding deficiencies in causes of death other than cancer in the towns of Group (*a*). Most of the causes which could be

TABLE III.—*Correlation Coefficients between Cancer of Stomach C.M.R., Social Class and Age Indices, and Hardness of Water Supply.*

	Males.					Females.				
	1st order.			For constant.		1st order.			For constant.	
	North.	South.	All.	S.C.I.	Age.	North.	South.	All.	S.C.I.	Age.
C.M.R. with social index	+·281	+·577	+·528	..	+·393	+·317	+·451	+·509	..	+·311
C.M.R. with age index	−·196	−·510	−·406	−·147	..	−·130	−·455	−·454	−·180	..
Social class with age index	−·706	−·470	−·577	..	..	−·732	−·585	−·663	..	..
C.M.R. with hardness	+·304	−·231	..	..	..	+·072	−·373	..	..	..
Social index with hardness	+·349	−·039	..	..	..	..	..	..	..	..

TABLE IV.—*Comparison between Cancer of Stomach Mortality at Successive Ages in Towns with High and Low C.M.R.*

	Males.						Females.					
	15–.	25–.	45–.	55–.	65–	75+.	15–.	35–.	45–.	55–.	65–.	75+.
Group (*a*)	125	129	126	122	119	118	129	121	131	131	128	120
Group (*d*)	73	64	63	76	77	85	67	68	67	69	72	83
Difference	52	65	63	46	42	30	62	53	64	62	56	37
Ratio (*a*)/(*d*)	1·71	2·01	2·00	1·61	1·55	1·35	1·93	1·78	1·96	1·90	1·78	1·45

TABLE V.—*Comparison between Cancer of Stomach Mortality at Successive Ages in Social Classes*, 1930–32.

	Males.						Females.					
Social class.	25–.	35–.	45–.	55–.	65–.	70+.	25–.	35–.	45–.	55–.	65–.	70+.
I–II	95	65	76	81	90	102	74	67	70	75	93	95
III	90	100	100	97	104	102	95	109	107	110	113	123
IV	119	115	107	114	104	104	100	105	105	108	104	133
V	129	121	126	120	118	110	126	116	126	127	125	130
Range	34	56	50	39	28	8	52	49	56	52	32	35
V per cent of I–II	136	186	166	148	131	108	170	173	180	169	134	137

TABLE VI.—*Correlation Coefficients between Cancer of Stomach, Peptic Ulcer and Other Digestive Disease* Death Rates in* 1931–39.

	Age group.	Males.			Females.		
		North.	South.	All.	North.	South.	All.
Cancer of stomach with peptic ulcer	45–64	−·152	+·059	−·066	−·347	+·028	−·185
	65+	+·270	−·235	−·006	−·066	−·106	−·169
Cancer of stomach with other digestive disease*	45–64	−·108	−·062	−·077	+·120	+·087	+·268
	65+	−·081	−·017	−·015	−·158	+·729	+·395

* Diseases of digestive system except peptic ulcer and appendicitis, but including cancer of liver not known to be secondary.

confused with cancer of the stomach, such as gastritis, gall bladder and liver diseases, including cancer of the liver, and intestinal diseases other than appendicitis are included in the group " other digestive diseases," whilst peptic ulcer has been dealt with separately. The total deaths in all the county boroughs during 1931–39 from the various groups of causes concerned, and from cancer of the stomach, at ages 45–65 and at 65 and over were as shown in Table VII.

TABLE VII.—*Deaths in County Boroughs from Cancer of the Stomach.*

Registrar-General's short list groups (1931–39).	Males.		Females.	
	45–.	65+.	45–.	65+.
Cancer of stomach	9995	9374	6381	9434
22. Peptic ulcer	5961	2067	1553	1154
25. Cirrhosis of liver	1370	878	730	437
26. Other diseases of liver, etc.	907	1035	2055	2796
27. Other digestive diseases	4097	4183	4211	5198
Cancer of liver	1383	1785	1199	2137
Total " other digestive "	7757	7881	8195	10,568

At the age-groups 45–65, 65 and over, the totals of cancer of stomach deaths were not much different from the totals of digestive diseases excluding peptic ulcer and appendicitis, and since the great bulk of any transfer of deaths into or out of the cancer group by wrong certification must pass out of or into the " other digestive " group, this means that a strong negative correlation must exist between death rates attributed to the two groups if the large variation in stomach cancer rates arises from diagnostic differences amongst the towns. Indeed, if this were the main cause, doubling the stomach cancer rate (as in Group (*a*) compared with Group (*d*) towns) would involve almost abolishing the rate for other digestive diseases. If there is no appreciable negative correlation the diagnostic factor cannot be of any importance as a cause of the variation. Transfer might also occur between stomach cancer and peptic ulcer as a result of mistaken diagnosis, but the numbers of deaths attributed to the latter group were small in comparison with those attributed to the former, except amongst males aged 45–65. It follows that the transfer of the whole of them to cancer, if that were possible, in certain towns would fail to account for the excess of certified cancer in those towns ; and even large negative correlations between the two causes could not account for the variation in stomach cancer amongst males over 65 or amongst females of either age group.

Table VI shows that for males of both age groups cancer of stomach death rates had no appreciable correlation with either peptic ulcer or other digestive diseases ; for females of both age groups there was a small but statistically insignificant negative correlation with peptic ulcer, and a small just significant positive correlation with other digestive diseases in the country as a whole.

Since none of the factors tested above can account for the differences in Tables I and II, the remaining explanation is that some extrinsic irritant factor is concerned which differed in intensity in the various towns. In order to test

Cancer of stomach with other digestive disease* . { 45– . . . −·062 . −·077 . +·120 . +·087 . +·268 / 65+ . . . −·081 . −·017 . −·015 . −·158 . +·729 . +·395

* Diseases of digestive system except peptic ulcer and appendicitis, but including cancer of liver not known to be secondary.

further the curious contrasts in cancer of stomach mortality in London boroughs when grouped according to their source of water supply (Registrar-General, 1947), correlation coefficients were calculated between the C.M.R. for cancer of the stomach during 1921–39 and the estimated total hardness of the water supply of the 83 towns during 1911–31, as shown in Tables I and II. It has to be remembered that the location of certain industries has been influenced to some extent in the past by the need for a soft water supply, and that in the north of England much of the industrial population is served by soft water. Of the 42 northern towns 23 had water supplies with less than 7 degrees, or 10 parts per 100,000 of total hardness; their average social class index was about 37 per cent unskilled and partly skilled workers. The 9 northern towns with water supplies of 20 or more parts per 100,000 total hardness had an average social class index about 42 per cent; and the correlation between hardness and social index was significantly positive. In the 41 towns elsewhere there was no correlation between the two indices.

The coefficients of correlation in Table III between cancer of stomach mortality and hardness were positive in the North for males and negative in the South for each sex; and when all the towns are grouped according to an ascending scale of water hardness, it appears that the C.M.R. was low in the groups with moderate hardness and higher in the towns with very soft or very hard water. No explanation is offered for this, but the figures are placed on record because they might at some future time fit in with other facts (Table VIII).

TABLE VIII.—*Comparative Mortality Ratios in Towns of Varying Water Hardness.*

Hardness per 100,000.		0–.	5–.	10–.	15–.	20–.	30 and over.	All towns.
Number of towns		15	16	16	13	15	8	83
C.M.R.	Males	108	93	110	88	92	102	100
			103					
	Females	112	97	110	85	91	100	100
			106					

Whatever irritant may be concerned in the production of gastric cancer, the effect of greater intensity of the irritant in one environment than in another might be (1) to increase the proportion of people developing cancer at each age without changing the latent period of development; (2) to shorten the average latent period before cancer appears without changing the proportions of people affected, or (3) to increase the proportion affected and also shorten the latent period. If the irritant is a living organism the average latent period is unlikely to vary, and the first effect would be expected from analogy with other infective processes. If it is a long-continued mechanical or chemical injury to cells the predominant effect to be expected from increased intensity of action would be a shortening of the latent period, without necessarily increasing the proportion of people affected, although that might also occur.

Differences in proportions of people affected in two localities without change in latent period would result in the same relative difference in death rate at each

age, provided the numbers dying were small in comparison with the living population. The ratios between death rates in groups of towns and between social groups would not be expected to fall with advancing age after 50 or 60 as in Tables IV and V, and this suggests that the irritant is not an infective organism.

Assuming some form of chemical or mechanical irritant is concerned, the latent period before cancer appears will vary rather widely about a mean value in the manner characteristic of most measurable vital factors ; and if the latent periods of gastric cancers in residents of all towns could be measured they would form a distribution approximating to the " normal " type.

It would be possible to reproduce the observed age-incidence of deaths from gastric cancer on the supposition that the irritant begins to affect a certain proportion, P, of people who are susceptible to it at about 15 years of age, and that at each year of age thereafter an additional proportion P/K become susceptible to it and begin to be affected. If, for example, the mean latent period before death were 20 years, with a standard deviation of 5 and normal distribution, K being a suitable constant, the numbers of deaths at successive quinquennial age periods from 25–29 to 65–69 would give a reasonably good fit to the deaths registered in England and Wales in 1947. A shortening of the latent period in the towns where the irritant was most intense and a lengthening of it in those towns where it was least intense would then result in the ratio between the respective death rates diminishing with advancing age after about 40, as in Table IV. The observed facts appear to be consistent with such an explanation, but a careful study of the mathematics of the assumptions outlined above is needed before definite conclusions can be reached.

SUMMARY OF CONCLUSIONS.

The death rates from cancer of the stomach in the 83 county boroughs of England and Wales in the period 1921–39 show great differences, which cannot be explained either by chance variations, or by differing accuracy of certification of the cause of death. Standardized mortality tends to be greater in the northern towns, in towns with a low proportion of people of advanced age and in towns with a high proportion of men in unskilled and partly skilled occupations. The contrast between death rates in the towns with high and low mortality diminishes with advancing age after about 45. Towns with a water supply of moderate hardness tended to have lower rates than towns with soft or very hard water. Cancer of the stomach death rates of men were unrelated with those for peptic ulcer or other digestive diseases ; but amongst women there was a small negative correlation with peptic ulcer and a positive correlation with other digestive diseases. The facts seem to be consistent with the hypothesis that gastric cancer depends on an irritant whose latent period is shorter in some towns than in others.

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THE DATA RELATING TO CANCER IN THE PUBLICATIONS OF THE GENERAL REGISTER OFFICE.

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THE compilation of this paper was prompted by the idea that some investigators of cancer as it occurs in man do not know the valuable material which is contained in the publications of the General Register Office, while even those who are acquainted with this literature may find some use for a list of the collections of data about cancer which it contains. The paper is intended only to indicate the kind of information which is to be found; no attempt is made to summarize the results.

The material dealt with in these publications consists of the death certificates from England and Wales, considered in association with the data on population, civil state, occupation and locality obtained at the Census of 1911, 1921 and 1931, together with population estimates for intervening and subsequent years. A death certificate gives the following particulars about the deceased person: name, sex, age, occupation, and date of death. Address at which death took place, and home address if different from this. District in which death was registered. Cause of death (primary and secondary). The certificate does not state, and is not intended to state, how long the deceased had followed the occupation named, and had lived at the home address given; this information could be obtained only by inquiry into individual cases. The laborious nature of such inquiries, which are impracticable on any large scale, can be seen in the monograph by Henry (1946).

Many writers have emphasized the high proportion of errors in the assignment of the cause of death (Willis, 1941, 1948, pp. 66–78), and unpublished material by Willis quoted by Kennaway and Kennaway (1947). The sources of such errors have been discussed in two earlier papers (Henry, Kennaway and Kennaway, 1931; Kennaway and Kennaway, 1936). One must either recognize these errors and correct them in any way possible, or abandon any attempt to obtain information from these data. The General Register Office sends out every year ". . . some 14,000 inquiries for clarification or fuller information concerning causes" of death (Stocks, 1950). Some evidence on the uniformity of error in death certificates is given below.

The Decennial Supplement (Occupational Mortality).

The Decennial Supplements for 1921 and 1931 contain data collected at the Census of those years, and were published in 1927 and 1938 respectively (Registrar-General, 1927, 1938). They contain a large amount of tabulated material for periods adjacent to the Census, namely 1921–23 and 1930–32; the scope of those tables, in so far as they are concerned with cancer, is summarized in Tables I and II.

The Decennial Supplement for 1931 (Registrar-General, 1938) shows for the first time data for the social incidence of cancer upon women, both married and

TABLE I.—*Tables in Decennial Supplement*, 1921 (*Occupational Mortality*). *Males*, 1921–23.

Table and page in Decennial Supplement.	Subject.	Ages.	Classes and occupations.	Cancer.
Table C, p. CXVIII	Standardized Mortality (C.M.F.) for all Causes and Certain Selected Causes	20–65	5 Social Classes and 180 Occupations.	All sites.
Table D, p. CXXIII	Same, compared with that of All Occupied and Retired Civilian males taken as 1000	Same	Same	Same.
Table F, p. CXXIX	Mortality Rank. Position of each Occupation in a List arranged for each of 23 Causes in order of Standardized Mortality from No. 1 (lowest) to No. 178 (highest)	,,	178 Occupations	All sites. Stomach.
Table G, p. CXXXII	Mortality from 41 Causes compared with that of All Occupied and Retired Civilians taken as 100	8 groups, 16– to 70–	5 Social Classes and Never Occupied	All sites.
Abstracts, p. 1	Deaths, and Mean Annual Death Rate per 100,000, from 43 causes	Same	5 Social Classes and 180 Occupations	All sites. Skin. Lip. Tongue, Oesophagus. Stomach. Other sites.
Table *d*, p. 125	Deaths of Non-Civilians, and of Men Retired, without record of civilian occupation, from Army, Navy and Air Force	,,	—	Same.
Appendix C, p. 129	Deaths, and Mortality per Million living, of all Occupied and Retired Civilians	,,	5 Social Classes	36 sites.*
Appendix D, p. 136	Standardized Mortality (C.M.F.), and Ratio to that of all Occupied and Retired Civilians taken as 1000	20–65	178 Occupations	Tongue. Oesophagus. Stomach.

* Lip. Tongue. Mouth. Jaw. Tonsil. Pharynx. Oesophagus. Stomach. Small Intestine. Caecum. Hepatic and splenic flexures. Sigmoid flexure. Colon. Rectum and anus. Gall bladder. Lung. Liver. Abdomen. Neck. Lymphatic glands. Mediastinum. Other sites.

TABLE II.—*Tables in Decennial Supplement, Part IIa*, 1931 (*Occupational Mortality*). *Males, and Married and Single Women*, 1930–32.

Table in Decennial Supplement.	Sex.	Subject.	Ages.	Classes and Occupations.	Cancer.
Table 4A	Males	Deaths	9 groups, 16–75–		
		Calculated Standard Deaths, and Registered per 100 Standard Deaths.	20–65 and 35–65	5 Social Classes and 88 Occupation Groups	All sites. Buccal cavity and Pharynx. Oesophagus and Stomach. Lung and Pleura. Skin.
4B	Married Women	Same	Same	Same. 44 (Husband's) Occupation Groups	Same. Uterus. Breast.
4C	Single Women	,,	,,	Same. 47 Occupation Groups	All Sites. Uterus. Breast.
4D	Males	Deaths	35–65 and 20–65	290 Occupations and Groups including Miners by Districts	All Sites. Buccal Cavity and Pharynx. Oesophagus and Stomach. Skin.
		Calculated Standard Deaths	20–65		
4E	Married Women	Same	Same	36 (Husband's) Occupation Groups	Same.
4F	Single Women	,,	,,	32 Occupation Groups	All sites. Uterus. Breast.
Table 5	Males, Married and Single Women	Comparative Mortality	20–, 25–, 35–, 45–, 55–65	5 Social Classes	All Sites.
Table 6A	Males	Same	20–65	5 Social Classes and 360 Occupations	Same as 4D.
6B	Married Women	,,	Same	5 Social Classes and 75 Occupations	Same as 4D.
6C	Single Women	,,	,,	5 Social Classes and 79 Occupations	Same as 4F.
6D	Males	Comparative Mortality	20–65	Extension of 6A	Lung and Pleura.
6E	Married Women	Same	Same	Extension of 6B	Lung and Pleura. Uterus. Breast.
Table 7		Mortality Rate of Males as per cent of that of Married Women of same Social Class or Occupation (husband's) Group	35–65	44 Occupations	Same as 4D.
Table 8	Males, Married and Single Women	Deaths. Rates per Million. Per cent of those at all sites	8 Groups, 16 to 70– 45–55, 55–65, 65–	5 Social Classes	41 Sites.†
Table 9A	Males	Deaths	6 Groups, 20– to 70– 45–65, 65–	39 Occupations and Occupation Groups	All Sites. Lip. Tongue. Mouth. Jaw. Oesophagus. Stomach. Large Intestine. Rectum. Pancreas. Larynx. Lung and Pleura. Prostate. Skin. Ethmoid and Nasal Bones.
		Site Deaths as per cent of those at all sites			
9B	Single Women	Same	Same	14 Occupations and Occupation Groups	All Sites. Stomach. Large Intestine. Gall Bladder. Uterus. Ovary. Breast Skin.

single. The 240,726 deaths of all married women during 1930–32 were classified according to the husband's occupation as stated on the death certificate, while 19,422 single women who died during the same period were classified according to their own occupations. The reasons for the association of the married woman with the occupation of her husband are that (1) " Only about 10 per cent of married women were recorded as gainfully occupied at the Census of 1931," and (2) some indication is obtained of the social, or occupational, nature of the mortality in the husbands' occupation, ". . . for no trade could longer be regarded as directly prejudicial to health if it were found to entail as much excess risk for the wife as for the husband. In such a case excess mortality would have to be attributed to the social conditions implied by the occupation . . ." (p. 3 of Decennial Suppl., 1931). This matter is dealt with further in the last section of this paper. Table IX shows a selection from the Supplements for 1921 and 1931 of some very interesting data for the incidence of cancer of various organs upon men and married women of the five social classes, and in the case of the men two periods (1921–23 and 1930–32) can be compared. A recent paper by Swanston (1950) on " The Iron and Steel Industry " shows the use which can be made of the material in the Decennial Supplements.

The Statistical Reviews.

The Registrar-General publishes annually a " Statistical Review of England and Wales for the Year . . .," normally in two parts, namely, (1) Text, (2) Tables. The information given in these Reviews about cancer has been amplified progressively during recent years. The " Text " now contains a section upon cancer consisting of a commentary upon the data which have been obtained during the year in question, and certain tables.

This section in the latest Review (1940–45, Text) deals with—

(1) *The total mortality from cancer in rates per million living.*

(*a*) By sex, and by age in periods (0–5, 5–, 15–, 25–, 35–, 45–, 55–, 65–, 75 and over) (Table LXXIV).

(*b*) In comparison with that in various earlier years, or groups of years (Table LXXV).

(*c*) When corrected by standardization to eliminate changes in the age structure of the population (p. 137). This table provides material in answer to the question so often asked—" Is cancer increasing ? " The Comparative Mortality Index, which takes account of changes in the sex and age structure of the population, shows that the total mortality from cancer in England and Wales has barely increased during the past twenty years.

FOOTNOTES TO TABLE II.

* Tables 4A, 4B and 4C give also, for Nine Age Groups: (*a*) Census Population; (*b*) Mean Annual Death Rate from All Causes (per 100,000); (*c*) Ratio of Death Rate to that of All Males (taken as 100).

† Lip. Tongue. Mouth. Tonsil. Jaw. Pharynx. Palate, cheek, salivary glands, gums. Oesophagus. Stomach. Small intestine. Caecum. Hepatic, splenic and sigmoid flexures. Large intestine. Rectum. Liver. Gall bladder. Pancreas. Peritoneum, etc. Larynx. Lung. Mediastinum. Uterus. Ovary. Vulva. Breast. Kidney, suprarenal. Bladder, urethra, ureter. Prostate. Testis. Penis. Scrotum. Skin. Brain, meninges. Thyroid. Bones. Ethmoid nasal bones. Other sites.

(*d*) Attributed to sarcoma, carcinoma and "cancer" undefined by sex and age-groups. The distinction between the entry of "carcinoma" and "cancer" upon death certificates is probably of no value, but the separation of the two at any rate absolves the General Register Office from any responsibility for the distinction (Table LXXVI).

(*e*) By regions (England and Wales, Greater London, Rest of South-East, North, Midland, East, South-West, Wales), and by class of area (county boroughs, other urban districts, rural districts) (Table LXXVII).

Such data for the total incidence of cancer must, of course, be recorded, calculated and examined, but they cannot show the peculiar incidence of cancer upon the various organs, and in the two sexes, and in persons living in different areas, and various interesting changes may cancel one another and hence be lost in the totals. Hence figures for the total mortality from cancer are not very instructive.

(2) *The mortality from cancer of different organs.*

(*a*) The tables show the numbers of deaths from carcinoma, sarcoma and cancer undefined in about 40 organs, in males and in females separately. The great interest of these data is best made plain here by reproduction in Table III of a part of an example (Table LXXVIII*a*, 1940–42).

TABLE III.—*Deaths from Cancer of Different Forms and Sites*, 1940–42.

	Males.			Females.		
	Carcinoma.	Sarcoma.	Undefined cancer.	Carcinoma.	Sarcoma.	Undefined cancer.
All sites	89,902	4,053	6,436	97,006	3,226	6,378
Lips	606	5	20	61	2	—
Tongue	2,254	2	155	391	3	21
Mouth	703	2	37	120	—	5
Tonsil	534	42	34	73	30	12
Pharynx	1,008	24	62	310	16	31
Jaw	748	147	77	274	95	20
Others	529	10	26	106	5	12
Total	6,382	232	411	1,335	151	101
Oesophagus	4,498	2	299	1,906	3	153
Stomach	20,778	17	1,123	16,348	13	893
Duodenum	129	2	19	106	—	12
Other small intestine	155	35	13	173	19	16
Caecum	860	5	32	1,199	5	70
Hepatic flexure, splenic flexure	418	1	28	443	—	21
Sigmoid flexure	2,157	1	99	2,316	3	97
Large intestine (colon)	8,801	9	442	11,219	11	566
Other and undefined intestine	228	11	49	275	7	68
Rectum (not anus)	10,365	4	562	6,682	7	312
Liver	2,581	34	308	2,803	24	302
Gall bladder and ducts	703	—	64	1,528	—	118
Pancreas	3,267	5	187	2,884	6	148
Peritoneum, mesentery, others	199	250	44	364	239	53
Other and unspecified digestive organs	—	—	—	1	—	—
Total	55,139	376	3,269	48,247	337	2,829

TABLE III.—*Continued*

	Males.			Females.		
	Carcinoma.	Sarcoma.	Undefined cancer	Carcinoma.	Sarcoma.	Undefined cancer.
Larynx, trachea	2,491	3	163	755	3	43
Lung, bronchus, pleura	11,302	203	802	2,877	74	265
Mediastinum	460	246	232	202	107	78
Total	14,253	452	1,197	3,834	184	386
Cervix uteri				5,482	3	162
Corpus uteri				721	7	33
Other and undefined uterus				6,416	173	510
Total				12,619	183	705
Ovary and Fallopian tube				4,952	76	499
Vulva and vagina				1,281	30	48
Other female genital organs				15	1	3
Total				6,248	107	550
Breast	163	8	11	20,049	58	1,114
Scrotum	155	3	6			
Prostate	6,055	19	593			
Testis	339	147	44			
Penis	457	5	24			
Others	3	—	—			
Total	7,009	174	667			
Kidney	409	606	93	312	452	92
Bladder, urethra, ureter	3,360	17	219	1,557	6	93
Total	3,769	623	312	1,869	458	185

The material contained in this table is very significant. The cancer problem in its simplest form is reduced to the question, why does a cell divide? But no single universal "cause of cancer," such as is announced from time to time, could, even if it were actually discovered, explain all the features of this table. While fundamental research on the nature of cancer proceeds by means of the most refined cytological methods, there is scope for investigation of factors occurring in everyday life which may explain the very peculiar sex- and organ-distribution of cancer.

TABLE IV.—*Deaths from Carcinoma*, 1940–42 (*from Table LXXVIIIa; see Table III*).

Males.		Females.	
Scrotum, prostate, testis, penis, etc.	7,009	Uterus, ovary, tubes, vulva, vagina, etc.	18,867
Breast	163	Breast	20,049
Total cancers of male reproductive organs	7,172	Total cancers of female reproductive organs	38,916
Other organs	82,730	Other organs	58,090
Total cancers in males	89,902	Total cancers in females	97,006
		„ „ males	89,902
		Excess in females	7,104

For instance (Table IV), the total deaths from carcinoma in males (89,902) and females (97,006) show a difference of only about 7 per cent, yet the latter total includes 38,916 deaths, or 40 per cent of the whole, from carcinoma of organs peculiar to the female, among which organs the female breast, being capable of lactation, must for the present purpose be reckoned. This calculation illustrates the phenomenon of the " amount " of cancer in man, to which attention was drawn in an earlier paper (Kennaway and Kennaway, 1937). No better example has been given by later workers than that recorded by the Dutch investigators Snijders and Straub (1924) in Sumatra, who first described this numerical relation of total cancers in populations differing in habitat or in sex (Table V).

TABLE V.—*The Total Incidence, and Organ Incidence, of Cancer in Different Countries (Snijders and Straub, 1924).*

	Mortality from cancer per 100,000 per year.	Primary carcinoma of liver per cent of all carcinomas.
Javanese in Sumatra—		
Men	26·6	84
Women	25·4	22
Dutch in Holland of same age-groups—		
Men	24·3	
Women	24·5	
England and Wales—		1931. 1943–45.
Men	..	4·8 2·8
Women	..	4·7 2·7

These figures show that in Sumatran and European populations, in which the total incidence of cancer is about the same, the proportion of one form of cancer, in this case that of the liver, may be very different. A given total " amount " of cancer may thus be distributed over the various organs in different proportions. " There appears to be a general law that when in a given population the incidence of cancer in one particular organ is markedly increased as compared with another population, there is then a compensating decrease in the incidence of cancer in a number of the other organs " (Cramer, 1936). This phenomenon appears not to have attracted the attention of statisticians.

(*b*) The data shown in Table LXXVIII*a* and *b* are subdivided under 15 quinquennial or decennial age-groups from 0–5 to 85– years (Tables LXXIX*a*, *b*, *c*, *d*, *e*, *f*).

(*c*) The deaths from cancer of 30 sites in males and females separately are shown as death rates per million living, in age groups 0–35, 35–, 45–, 55–, 65–, 75 and over during the periods 1911–20, 1921–30, 1931–35, 1936–39, 1940–44, 1945 (Table LXXX). This table contains a very large amount of information on the changing incidence of cancer in so far as this is revealed by death certificates. The following examples taken from this table show various types of change since 1911 (Table VI). Thus in men aged 55–64 the mortality from cancer of the lip has fallen to 26 per cent of the initial figure, while that attributed to cancer of the lung has increased by more than 17 times.

TABLE VI.—*Changes in Frequency of Cancer of Various Organs*, 1910–1945.

Year.	Males. 0–35 *E.A.D.R.	35–	45–	55–	65–	75+	Females. 0–35 *E.A.D.R.	35–	45–	55–	65–	75+
Lips.												
1911–20	0	2	10	38	108	335	0	0	1	2	5	21
1921–30	0	1	7	36	104	297	0	0	1	2	6	19
1931–35	0	1	7	27	90	281	0	0	0	1	5	15
1936–39	0	1	4	21	74	250	–	0	0	1	4	19
1940–44	0	1	3	16	65	194	–	0	0	2	4	15
1945	0	–	1	10	48	177	–	–	–	2	3	10
Lung, Bronchus, and Pleura.												
1911–20	3	13	34	64	76	42	1	8	20	34	42	28
1921–30	3	29	73	128	136	92	1	10	25	50	60	51
1931–35	5	69	217	350	347	239	2	18	47	94	124	105
1936–39	8	99	335	579	570	381	2	24	60	123	176	155
1940–44	8	129	453	866	799	498	3	27	72	140	205	183
1945	10	144	555	1116	1050	571	4	29	79	169	238	200
							Females.					
	Breast.						*Uterus.*					
1911–20	6	176	474	696	946	1503	11	218	556	791	861	773
1921–30	7	187	508	782	1052	1740	11	200	473	682	831	823
1931–35	8	189	529	839	1098	1849	8	171	414	585	742	745
1936–39	8	184	516	830	1132	1889	7	151	386	557	697	747
1940–44	9	201	533	823	1114	1612	7	127	377	556	684	690
1945	9	198	500	784	1067	1590	7	98	340	541	629	688

* E.A.D.R. = Equivalent Average Death Rates.

The exact comparison of the prevalence of cancer, or of any form of cancer, at different periods, requires Standardized Death Rates owing to the continual increase in the numbers of older persons, who are of "cancer age." Such data are given at intervals in the Statistical Reviews (Text), and most recently in Table LIX in the Review for 1938 and 1939 (Registrar-General, Statistical Review), which records the standardized rates per million population for cancer of 27 sites in men and women separately in the periods 1901–10, 1911–20, 1921–30, 1931–35, 1936, 1937, 1938 and 1939. These figures for each one of the four most recent years are of especial interest because they show the present state of increase or decrease of the various forms of cancer. In the table in question the great majority of the 52 sex-site combinations show a decrease, or are stationary; the only quite definite increase is in cancer of the lung and bronchus in both sexes. Since 1942 Standardized Death Rates have been superseded by Comparative Mortality Indices, which compare the death rate in each year with that of 1938, after making allowance for changes in the proportion of the population at different ages. The annual C.M.I. for a number of sites of cancer since 1933 have been published in the Medical Tables volume of the Statistical Review (Table VIII) for each year since 1942.

(*d*) The deaths from cancer of certain sites (mouth and oesophagus, stomach and duodenum, respiratory system, breast, uterus, other sites) per 1000 for all sites in men and women at certain ages, in certain regions and classes of areas named above (Table LXXVII) during 1940–42 and 1943–45 (Table LXXXI) are set forth. This table is of great interest, as it provides a comparison between rural, urban and predominantly industrial (North, Midland) areas. The high

TABLE VII.—*Deaths from Cancer of Certain Sites per* 1000 *for all Sites, at Certain Ages, in Regions and Different Classes of Area (Civilians),* 1940–42 *and* 1943–45. (*Statistical Review,* 1940–45, *Text, Table LXXXI.*)

Cancer of Respiratory System.

			England and Wales.	Greater London.	Remainder of South-East.	North.	Midland.	East.	South-West.	Wales.	County boroughs.	Other urban districts.	Rural districts.	
A	Males, all ages—	1940–42	156	216	145	155	148	106	101	114	173	133	103	= 100
		1943–45	183	249	169	181	171	131	127	143	199	159	126	
	Females, all ages—	1940–42	41	51	37	43	37	35	33	38	42	37	36	
		1943–45	45	55	42	47	41	38	30	39	46	41	39	
B	Per cent of rural districts	Male	151	210	141	151	144	103	98	111	168	129		
		1940–42	145	198	134	144	136	104	101	113	158	126		
		Female	114	142	103	119	103	97	92	106	117	103		
		1943–45	115	141	108	121	105	97	77	100	118	105		

incidence of cancer of the stomach in both sexes in Wales, discovered by Stocks (1936), is well shown.

The cancers of the respiratory system show the greater liability of urban populations (Stocks, 1936); this portion of the table is of such interest that it may be reproduced *in extenso* here (Table VII, Section A).

These figures provide material for a calculation of some interest. If one recalculates the figures for the various districts on the basis that "Rural Districts" = 100, whereby the data for the two periods 1940–42 and 1943–45 become more comparable, one obtains the figures shown in Section B of the above table, which show a very close similarity between the two series for women, with the single exception of the figures for the South-West region. Of course this result has no bearing on the accuracy of death certificates, but it does show that two sets of such certificates, gathered in a number of districts during two consecutive periods, give results which have a considerable uniformity.

When the mortality from any form of cancer, such as that of the lung, is higher in urban than in rural districts, one must consider whether the difference is due, not to the conditions of life in towns, but to better facilities for diagnosis, and a further extract from Table LXXXI is of interest in this respect (Table VIII).

TABLE VIII.—*Deaths from Cancer of Certain Sites per* 1000 *for all Sites in Certain Areas.*

Cancer.		England and Wales.	Greater London.	Rural districts.
Males, 45–65				
Mouth and oesophagus,	1940–42	92	90	100
	1943–45	73	68	84
Stomach and duodenum	1940–42	238	205	254
	1943–45	225	187	235
Males, all ages—				
Respiratory system	1940–42	156	216	103
	1943–45	183	249	126
Females, 45–65				
Breast . . .	1940–42	236	261	236
	1943–45	231	254	244
Uterus . . .	1940–42	165	150	148
	1943–45	157	137	144

The diagnosis of cancer of the oesophagus, stomach and duodenum, breast and uterus may be by no means easy, yet these forms of cancer do not show the difference seen in cancer of the respiratory system. This last must be made up largely of cancer of the lung, and hence these data provide further evidence of a carcinogenic factor in urban environment.

The annual Statistical Reviews do not deal with the social and occupational incidence of cancer, as the occupational distribution of the population is ascertained only at census periods.

In this summary of the Annual and Decennial publications of the General Register Office in relation to cancer one cannot of course refer to the numerous passages which treat of the material tabulated.

The Social Incidence of Cancer.

The Decennial Supplement for 1921 (Registrar-General, 1927) contains the first detailed study of the social incidence of cancer upon the various organs in men, which study developed from an earlier investigation of the incidence of cancer as a whole (Stevenson, 1923). In the publications of the Registrar-General " each census unit of occupation has been assigned to one of five graded Social Classes . . . after consultation with the Ministry of Labour. So far as is possible from the material available, Class I purports to represent the professional and generally well-to-do section of the population, Class III, skilled artisans and analogous workers, and Class V, labourers and other unskilled callings, while Classes II and IV are intermediate, comprising occupations of mixed types, or types not easily assignable to the classes on either side." The parts of the body affected by cancer were divided into two classes, namely : exposed sites (buccal cavity, pharynx, oesophagus, stomach, larynx, skin), and other sites.

The mortality from cancer of the exposed sites increases from Social Class I to Class V, while cancer of the other sites shows no such relationship (Table IX).

The author of the Decennial Supplement, 1921, comments upon these data : " It thus appears that a large proportion, at least, of cancer mortality is of a highly preventable nature, for we must suppose that if the conditions of life of all sections of society could be assimilated to those of its upper ranks, mortality from cancer of the exposed sites would fall for all classes to the Class I level. Indeed it is very possible that knowledge of the preventable causes accounting for the difference might provide the means of reducing if not eliminating these forms of cancer for all classes, for these causes might well be found to apply in varying degrees to all sections of society " (p. xxi).

The paragraph made up by these two sentences should be regarded as one of the classics of cancer research, for it brings carcinogenesis in man into relation with factors in everyday life which can be investigated, while the most refined methods of physics and chemistry are applied to the problem of cancer in general.

The most extreme instance of a social factor in the incidence of cancer appears in the case of cancer of the scrotum (Kennaway and Kennaway, 1946), which could in all probability be eliminated altogether by cleanliness, as is cancer of the penis by circumcision in infancy. In 1930–1–2 Social Class I, numbering 278,367 males, did not produce a single case of cancer of the scrotum (Registrar-General, 1938, Part II*a*, p. 350).

The figures (Table IX) for cancer of the upper alimentary canal in males show a significant reduction in the steepness of the social gradient from 58, 80, 99, 102, 140, to 63, 80, 97, 109, 129 ten years later. This may be due in part to " earlier or more effective treatment than before of cancer of the more accessible sites " in men of the poorer classes, as is suggested in the text, but probably also to the improvement of social conditions in other ways. The change has been chiefly in the cancers of the buccal cavity (tongue, tonsil, pharynx), for cancer of the oesophagus shows no, and that of the stomach very little, loss of gradient. The steeply graded figures for the larynx, in contrast to those of the buccal cavity, show no change, while those for cancer of the skin show a flattening which one would associate with improved personal cleanliness.

Figures for the social distribution of cancer in women in 1921–23 are not available, but the comparisons of those for men, and women, in 1930–32 are

TABLE IX.—*Standardized Mortality Ratio* at Ages 35–65 in each Social Class from Cancer of Various Sites.*
(*Decennial Supplements*, 1921 *and* 1931.)

Social class.	Males. 1921–23.					Males. 1930–32.					Married women. 1930–32.					Single women. 1930–32.				
	I.	II.	III.	IV.	V.	I.	II.	III.	IV.	V.	I.	II.	III.	IV.	V.	I.	II.	III.	IV.	V.
All sites	80	92	99	96	123	83	92	99	102	114	97	97	102	95	105					
Lip	30	50	70	140	170	—	56	68	147	183										
Tongue	48	73	95	100	165	78	60	98	110	143	—	(74)	105	144	(100)					
Tonsil	25	88	94	106	163	(86)	76	93	97	147										
Pharynx	89	78	100	160	161	(76)	83	99	87	130										
Oesophagus	76	91	104	88	130	74	87	98	94	130	(95)	84	101	95	116					
Stomach	60	82	100	106	130	55	83	98	112	122	49	77	105	106	121					
Upper alimentary canal	58	80	99	102	140	63	80	97	109	129	56	78	105	106	119					
Lower " "	105	105	100	93	98	98	103	102	100	95	102	101	103	90	103					
Larynx	72	96	93	96	135	60	81	98	90	143	(55)	115	95	104	102					
Skin	63	73	100	120	150	(59)	75	95	116	133	—	86	88	114	131					
Lung	100	109	97	79	124	107	96	101	91	112	(100)	100	110	82	91					
Breast											138	116	103	84	82	98		105	90	78
Ovary											143	116	102	77	83					
Uterus											65	78	99	106	130	88		110	127	

* Registered per cent of calculated deaths.

interesting. Cancers of the buccal cavity and pharynx show a steep social grading in men, but in married women there is no consistent variation between Classes III, IV and V, and a similar description would apply fairly well to cancer of the larynx (see Tables, pp. 39, 44) and oesophagus; cancer of the stomach, on the other hand, shows no difference in gradient. " . . . the social gradation of gastric cancer mortality can have little to do with the effects of occupation, and must arise from factors of selection, economics or environment which affect the wives of the men in occupational groups as much as they affect the men themselves." Cancer of the skin also affects men, and married women, of the different social classes in much the same way; ". . . the employment of a proportion, largest no doubt in Classes IV and V of married women in textile occupations, may be partly responsible for the mortality gradient for skin cancer."

No attempt is made here to deal with all the indications for inquiry which may be drawn from this table; the subject is dealt with, and illustrated by graphs, in pp. 33–49 of the 1931 Supplement. Comparisons between the earlier and later periods in men, and between men and married women in one of these, are often suggestive. Cancers of the buccal cavity, and of the larynx, show a high incidence upon occupations associated with alcohol (Kennaway and Kennaway, 1936; Wassink, 1930; Registrar-General, 1927, Appendix D); the change in gradient in the former only might suggest that this is due to another factor, namely, better oral hygiene.

The lack of influence of social class upon the liability to cancer of the lung, together with the very considerable effect of urban conditions (Stocks, 1936; Registrar-General, 1947, Table VII), suggests some carcinogenic factor to which all classes are exposed; owing to the mixing action of the wind there is less difference in the outdoor air breathed by different classes than in other social conditions such as food and cleanliness (Kennaway and Kennaway, 1947), and all classes seem to share in the increased use of tobacco, largely in the form of cigarettes.

A good example of the unique value of such studies of social distribution is given by cancer of the uterus, which was examined in this way for the first time in 1930–32. The figures in Table X are based upon 7831 cases of cancer of the uterus in married women and 1,294 cases in single women. Thus, at ages 35–65 the rate in married women of Class V is twice that of Class I, and single women in Classes IV–V show a mortality 44 per cent greater than that of Classes I and II.

TABLE X.—*Cancer of the Uterus. England and Wales*, 1930–32. (*Statistical Review*, 1936, *Text*, *p.* 90.)

Social class.	Mean annual death rate per million married women at ages:			Standardized mortality ratio (registered per cent of calculated deaths) at 35–65.		Per 1000 deaths from cancer of all sites at ages 65 and over.	
	35–	45–	55–65.	Married.	Single.	Married.	Single.
Class I	119	264	469	65	88	78	62
,, II	144	348	515	78		96	
,, III	197	438	635	99	110	102	76
,, IV	239	466	627	106	127	110	95
,, V	294	591	754	130		110	
All	209	445	519	100		103	

The gradient of fertility shows a somewhat similar relation to social class. Thus in 1921 the ratio of registered to 100 calculated births for married males in the five social classes was 85, 85, 97, 109, 128, and the social distribution of uterine cancer in married women might be thought to be due to the difference in fertility. But the data for single women show that the matter is not so simple. " There must exist factors closely bound up with the social class differentiation, either selective or environmental, or both, which are productive of uterine cancer, quite apart from the parturient histories of the women concerned " (Registrar-General, 1938, p. 48).

The social distribution of cancer of the breast, and of the uterus, in married women show an almost exactly inverse relationship which is especially obvious in the mirror-image appearance of the graphs (Registrar-General, 1938, diagram 4, p. 35). The similarity of figures for cancer of the breast, and for the much less common cancer of the ovary, is noteworthy.

The data on the mortality from cancer in the last twenty years should yield very valuable information when correlated with the results of the Census in 1951, the first made since 1931, in view of the considerable social changes which have taken place.

Other Publications.

(1) The British Empire Cancer Campaign has published in its 13th Annual Report a very elaborate study by Stocks (1936), illustrated by 17 maps, of the distribution of cancer of eight sites (oesophagus ; stomach ; intestines ; rectum ; liver, gall-bladder and pancreas ; skin ; lung ; breast) in both sexes at various ages in the counties and county boroughs of England and Wales. The 14th Report of the Campaign records an extension of this study (Stocks, 1937), with 7 maps, to cancer of other sites (tongue, mouth, jaw, larynx, bladder, prostate), and a final section (Stocks, 1939), with 9 maps, deals with cancer of the uterus, vagina, ovaries and Fallopian tubes, and of the skin, lung, rectum and bones in women. No such study of cancer in relation to this, or any other, geographical area has ever been made, and no summary is attempted here of the results and suggestions for research which it contains.

(2) The changes in mortality from cancer of various organs in England and Wales between 1911 and 1944 are described in a paper by Stocks and MacKay (1946).

(3) The General Register Office has published a study by Stocks (Registrar-General, 1947) of cancer of the stomach, oesophagus, lung, larynx, breast, uterus, ovary and some other sites in relation to a number of localities and environmental factors (urban and rural districts, counties, the 13 largest cities in England, 30 large towns, Metropolitan boroughs of London, social indices, number of persons per room, amount of sunshine, source of water supply) during one or other or both of two periods, 1921–30 and 1940–44. One may mention here two results which provide valuable indications for further research, namely (*a*) an inverse relationship between number of sunshine hours and deaths from cancer of the lung, and (*b*) a high incidence of cancer of the stomach in the northern and central counties of Wales, especially in the former. The author says : " The purpose of this report is to present the statistical facts, and point out any peculiarities in distribution and correspondences with other measurable factors which appear, so that all possible clues may be followed up by further study. Cancer is a grave

problem, and no effort should be spared to investigate every possible clue, no matter how unlikely it may seem at the moment that it will prove fruitful."

SUMMARY.

A list has been compiled of the types of information about cancer in man in England and Wales which are to be found in the publications of the General Register Office.

I wish to thank the British Empire Cancer Campaign, the Anna Fuller Fund, and the Jane Coffin Childs Memorial Fund for Medical Research for grants. I am greatly indebted to members of the staff of the General Register Office for instruction upon several questions which have arisen during the compilation of this paper.

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THE ARSENIC CONTENT OF TOBACCO AND OF TOBACCO SMOKE.

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PREVIOUS INVESTIGATIONS.

Two recent investigations, both from the United States, upon the arsenic content of tobacco, and the volatilization of this arsenic when tobacco is smoked, have been reported. The work of these authors differs from our own in that they used artificial methods of combusting the tobacco, which may or may not reproduce the conditions present when smoking is carried out in the ordinary way, and they found no brands of tobacco containing only minimal quantities of arsenic.

The Arsenic Content of Tobacco.

(1) Gross and Nelson (1934) employed a method which consisted of digestion in nitric and sulphuric acids, precipitation of magnesium ammonium arsenate, solution in hydrochloric acid, and estimation by the Gutzeit method. "The analyses were made on analytical units composed of either 1 cigar, 5 cigarettes, or 5 g. smoking tobacco. Five such analytical groups were analysed for each brand studied." They found the content of pooled groups of 4 cigarettes of 5 brands to range from 9·7 to 36·3 p.p.m. (Table I), while individual cigars showed a wider range (8·3 to 48·4) and pipe tobacco a smaller one (26·0 to 50·0).

(2) Thomas and Collier (1945), using a modified Cassil-Wichmann method, found that "individual cigarettes and cigars of a given brand varied widely in arsenic content," which ranged in pools of 2 to 4 cigarettes from 2 packs of 20 (presumably of the same brand) from 35·4 to 114 p.p.m., while cigars (13·2 to 29·5) and pipe tobaccos (22·7 to 42·8) showed a lower range (Table I).

Thus the cigarettes used in these two investigations showed nearly a 12-fold range (9·7 to 114 p.p.m.) in mean values. Gross and Nelson (1934) attribute the rather narrower range found in cigarettes and in pipe tobacco, compared with cigars, to the mixing which the former receive, but this difference is not seen in the results for cigarettes and cigars of Thomas and Collier (1945).

The Arsenic Content of Tobacco Smoke.

Apparently the first estimates of the arsenic volatilized from tobacco were made by Remington (1927), who found 6 to 30 p.p.m. in American pipe and chewing tobaccos; using suction by a water-pump, he concluded that "we can say roughly that half of the arsenic is evolved in the smoke . . ."

Gross and Nelson (1934) used an apparatus drawing about 50 ml. of air through a cigarette, cigar or pipe at the discharge of a syphon which took place

TABLE I.—*Arsenic Content of Tobacco.* (*Gross and Nelson*, 1934; *Thomas and Collier*, 1945.)

	Brand.	As_2O_3 parts per million.	Means of brands
Gross and Nelson:			
Cigarettes		Range in 5 pooled batches of 4 cigarettes each:	
	1	20·7 to 25·6	22·9
	2	21·1 „ 26·7	24·1
	3	25·3 „ 36·3	33·2
	4	9·7 „ 13·0	11·6
	5	22·9 „ 26·1	24·7
Cigars		Range in 5 single cigars from each of 5 brands:	
	1–5	8·3 to 48·4	11·6 to 26·6
Pipe tobacco	1–4	Range in 5 samples from each of 4 brands: 26·0 to 50·0	31·1 to 45·1
Thomas and Collier:			
Cigarettes	"A single pack of 20"	"Groups of 2 to 4." 35·4; 40·0; 40·5; 47·2; 60·0; 61·4*	..
	"Another pack"	61·4; 67·0; 71·4; 84·5; 114·0	..
Cigars (about 10% cut off at ends)	1–4	13·2; 22·5; 26·2; 29·5	..
Pipe tobacco	1–5	39·6; 26·5; 22·7; 34·5; 42·8	..

* These figures represent the actual analyses in p.p.m., on pooled groups of 2 to 4 cigarettes, and hence are not calculated means, though each figure represents the mean composition of the cigarettes in one pool.

three times a minute. Cigars and cigarettes were "smoked down to about 1/2 in. to 3/4 in. . . . ."; the arsenic in the "puffed" and "unpuffed" smoke was absorbed and estimated separately. The mean distribution of the arsenic recovered in the smoke, ash and butts is shown in Table II. Pipe tobacco was combusted until no residue but ash was left, while about 22 per cent of the cigars and cigarettes remained in the butts. The fraction in the puffed, as against the unpuffed, smoke is high in the case of pipe tobacco, which difference is attributed to "the dying down of the ember between puffs."

TABLE II.—*Arsenic Content of Tobacco Smoke.* (*Gross and Nelson*, 1934; *Thomas and Collier*, 1945.)

	Mean distribution of arsenic recovered.					Arsenic volatilized.		Tobacco smoked
	Unpuffed smoke.	Puffed smoke.	Total smoke.	Ash.	Butts.	Range.	Mean.	
	1.	2.	3.	4.	5.	6.	7.	8.
	%	%	%	%	%	%	%	%
Gross and Nelson:								
Cigarettes, 5 brands	12·3	11·0	23·3	48·6	28·1	32·2–41·3	36·1	ca. 75
Cigars, 5 brands	5·6	5·5	11·1	60·6	28·3	15·1–34·7	23·0	
Pipe tobacco, 4 brands	4·6	25·9	30·5	69·5	..	26·1–32·8	30·6	100
Thomas and Collier:								
Cigarettes, 6 brands:								
1st series	..	14	..	66	20	..	..	85
2nd „	5	14	19	63	18	..	..	87
3rd „	4	10	14	49	37	..	..	68
Cigars, 4 brands:								
1st series	4	7	11	62	27	..	..	72
2nd „	7	8	15	77	8	..	..	90
3rd „	2	12	14	79	7	..	..	90
Pipe tobacco, 4 brands	3	20	23	77	..	..	..	100

Gross and Nelson (1934) devote a good deal of attention to the arrest of volatilized arsenic in the butts, indicated by the amounts assumed to be present in the corresponding portion of the original cigar or cigarette. By such calculations they arrive at the figures given in the 6th and 7th columns of Table II, which are, of course, higher than those in Column 3. "The pipe smoker who smokes all the tobacco does not therefore have the protection afforded by the butts of cigars and cigarettes." From the present point of view this question is not of great interest; the important datum is the amount of arsenic inhaled, and in this country only about one smoker in ten smokes a pipe (*Hansard*, 1945).

Thomas and Collier (1945) used an apparatus similar to that of Gross and Nelson (1934), which pumped 10 to 50 ml. air during 1–2 seconds at 10-second intervals. In their first series (Table II) cigarettes of 7 cm. length were smoked down to 1 cm.; in a later series only 2/3 was burned. At first ". . . the arsenic found was compared with analyses of whole cigarettes from the same pack," but in view of the large differences they later cut off about 0·8 cm. for analysis; apparently they failed to discover that this method also can be fallacious. The first and second series show very little difference in the percentages although in the first 10–20 ml. and in the second 50 ml. was drawn through at each puff. The results are in general agreement with those of Gross and Nelson (1934), whose cigarettes contained considerably less arsenic (Table I).

METHODS.

Gutzeit Method.

Our earlier results (Table IV) were obtained by the Gutzeit method, which depends upon the brown and yellow colours produced in paper impregnated with $HgCl_2$; it is fitted for the measurement of amounts of As_2O_3 between 10 and 1 μg., and shows differences most clearly in the range of 8 to 3 μg. There may be considerable differences in the matches made by different persons; in these experiments the mean was taken of the readings made independently by two persons unaware of the nature of the unknowns. The iodometric method of Thomas and Collier (1945) was adopted later (Tables III, V and VI) in view of the difficulty of determining the small amounts of arsenic lost in smoking. The method has the advantage that, whatever may be its errors in other respects, there is less disagreement over the very sharp end-point (titration of iodine and starch), about which two observers usually do not differ by more than 0·05 c.c. (0·5 μg.), while in the Gutzeit method greater differences are common.

Before we had observed the differences in arsenic content which may be found within a single cigarette, three successive pieces were cut from a cigarette of Brand C—A weighing 0·1499 g., B 0·1527 g. and C 0·1429 g. To the middle piece, B, was added 1 ml. of the standard solution = 10 μg. As_2O_3. Gutzeit estimations upon the whole of A and C and upon 1/2 of B gave about 7 μg. in each one, hence, if this cigarette happened to be of fairly uniform composition, about 14 μg., or 82 per cent out of 17 μg., was recovered. Another test of the Gutzeit method is described under "Other Brands" below.

Method of Thomas and Collier (1945).

The estimations upon Brands P and C were usually carried out upon amounts of 20 to 30 μg., as a method which is sufficiently accurate within this range

allows of duplicate estimations upon single cigarettes containing 40 to 60 μg. The method and apparatus of Thomas and Collier (1945) were tested, using the standard solution of As_2O_3 (10 μg. in 1 ml.) either alone or added to an aliquot part of a solution containing a combusted cigarette, or ash and stump (Table III).

TABLE III.—*Percentage Recovery of Arsenic by Method of Thomas and Collier* (1945).

Arsenic, μg.	100	50	30	20	10
Standard solution alone :					
Number of estimations	11	2	4	6	4
Range	90·2–99·0	85·0, 94·0	87·4–92·4	90·0–107·5	98·0–105·0
Mean	94·6	89·5	90·3	97·5	100·25
Standard solution + combusted cigarette or ash and stump :					
Number of estimations	..	..	..	6	5
Range	..	..	..	77·5–88·5	74·0–100·0
Mean	..	..	..	83·2	87·0

With the pure solution, the results were less accurate with the higher amounts (30 to 100 μg.) than with the lower (10 to 20 μg.), though with the latter the titration error is of course considerable, as 0·05 ml. = 0·5 μg. Cassil and Wichmann (1939) claim " . . . an average recovery of 99·5 per cent " of amounts from 5 to 500 μg. In the presence of the combusted products the recovery is less complete (between 80 and 90 per cent). Gross (1933) has drawn attention to the occurrence of such errors in the estimation of arsenic in tobacco, which are attributed to uncombusted residues of pyridine and nicotine ; we failed to obtain satisfactory results by the method which he devised to overcome this difficulty. Fortunately the defect was of the same order with both the smoked and unsmoked material, so that the measurement of the arsenic volatilized was not affected.

ARSENIC CONTENT OF CIGARETTES, AND OF ASH AND STUMP.

In the previous investigations summarized above artificial methods of smoking were employed, air being drawn through a pipe, cigar or cigarette by means of a water-pump. This process might be fallacious, as there is no warrant that the apparatus reproduces the conditions present when tobacco is smoked in the usual way. We have confined our experiments wholly to cigarettes smoked by one or other of three persons accustomed to this form of smoking, and they were asked to do this in their ordinary way ; the two brands used (P and C) are in common use in this country. The ash, and stump, were dropped into separate weighing bottles in our earlier experiments, but latterly the two have been analysed together ; the amount of arsenic found, if less than that in the cigarette, should indicate the amount volatilized in the smoke, of which a part must have been inspired. But actually the problem is by no means simple, the difficulty being to ascertain the arsenic content of the cigarette originally (Table IV).

The data in Table IV show that the amount of arsenic (As_2O_3 μg./g.) in whole cigarettes, or in portions of these, from 5 packages of one brand (P), ranges from 24 to 106, and in one package from 56 to 106, while Brand C shows a smaller range (28 to 61). Hence it is impossible to infer the arsenic content of cigarettes about to be smoked unless the average of a very large number is ascertained. Before this width of range was realized we obtained many contradictory results, showing amounts in the ash and stump which were sometimes less, and sometimes more, than that thought to be present originally.

TABLE IV.—*Range of Arsenic Content in Two Brands of Cigarettes. Gutzeit Method.* (*See also Tables V and VI.*)

	Package and number of cigarettes.	As_2O_3 μg./g. Range.	Mean.
Brand P.			
8 Whole cigarettes	A 100	49·5–76·6	67·8
9 Cigarettes (7 wholes and 2 halves)	B 20	55·9–106·2	82·3
5 Pieces from 3 cigarettes	C 10	27·2–34·3	30·7
1 Piece from each of 4 cigarettes	D 20	35·8–59·7	49·6
6 Successive pieces cut from 1 cigarette	D 20	58·4, 51·9, 41·5, 58·4, 58·7, 38·8	46·1
13 Estimations on mixed tobacco of 10 cigarettes	E 10	23·9–31·9	27·5
Range in 5 packages of Brand P	..	23·9–106·2	..
Brand C.			
10 Pieces from 5 cigarettes	A 20	33·4–61·5	51·3
5 Successive pieces cut from 1 cigarette	A 20	54·2, 43·1, 51·8, 30·6, 51·0	46·1
11 Estimations on mixed tobacco of 10 cigarettes.	B 20	28·3–50·3	38·5
Range in two packages of Brand C	A and B 40	28·3–61·5	..

These results are reckoned as μg./g. in order to give a uniform basis of comparison. But as the weight of most cigarettes does not differ very much from one gram the figures give a rough measure of the arsenic in one cigarette, which is of more obvious practical interest. A quantity expressed as μg./g. is numerically the same as parts per million.

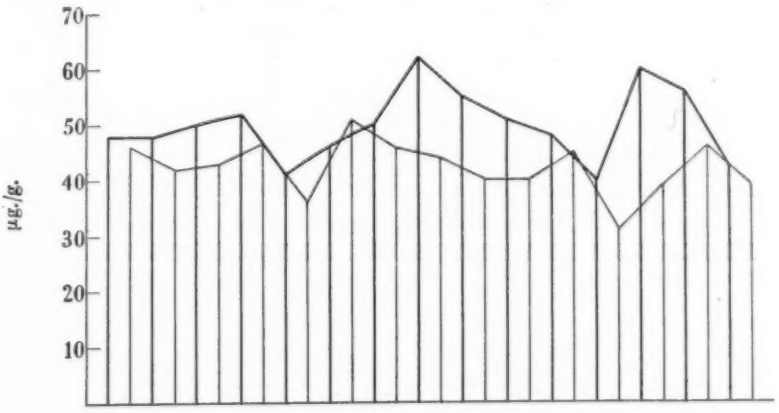

FIG. 1.—Consecutive analyses of cigarettes, and of ash and stump. Brand P.
———— Cigarette.
———— Ash and stump.

The Gutzeit method enables an estimation to be made upon 1/5 to 1/10 of a cigarette, such as those of Brands P and C. We therefore cut off such portions for analysis from cigarettes, of which the remainder was smoked in the manner described. The results were again contradictory, and we found that such successive portions may show a range of 38·8 to 58·7, or 100 : 151 in P, and of 30·6 to 54·2, or 100 : 177 in C (Table IV).

In the hope of obtaining more uniform material, the papers were removed from the cigarettes of a package of 10 or 20, and the tobacco mixed by hand and

minced with scissors. Cigarettes were made by rolling this tobacco in papers in a machine, a filter-plug being inserted at one end to keep the contents from falling out. But even this treatment does not give satisfactory results; it is very difficult to take up samples of such material which will contain constant proportions of the coarser and finer particles, as the latter tend to fall away from the former; the tobacco would have to be ground to a powder, similar to snuff, to secure uniformity. The analyses ranged in the ratio of 100 : 133 in P and of 100 : 178 in C. Moreover, the minced tobacco, even when moistened, is not easy to smoke in the same way as ordinary tobacco.

Adjacent cigarettes of Brand P from a much larger package, containing 100, were taken, but these at first showed a wide range (49·5 to 76·6, Table IV). After the adoption of the method of Thomas and Collier (1945), in place of that of Gutzeit, we came upon a row in this box of more uniform composition; this change cannot be accounted for by the greater accuracy of the former method. Successive single cigarettes or pairs were combusted as such, or smoked, alternately. The analyses of 30 consecutive cigarettes (Table V and Fig. 1) showed

TABLE V.—*Consecutive Analyses of Cigarettes, and of Ash and Stump. Brand P. Method of Thomas and Collier* (1945).

	As_2O_3 μg./g.	
	Cigarette.	Ash and stump.
	48·3	46·7
	48·6	41·1
	51·3	43·0
	52·7	47·1
	41·3	35·4
	46·7	51·7
	50·5	47·0
	62·9	44·7
	55·8	40·5
	51·8	40·5
	48·2	45·2
	40·2	30·8
	60·5	39·6
	56·1	46·0
	42·7	39·2
Mean	50·5	42·6

Loss per cent. = 15·8. P = 0·001.

that an average of 15·8 per cent of the arsenic is lost in the process of smoking and had presumably been volatilized; this loss is statistically significant (P = 0·001). The same method was applied to Brand C, of which the largest boxes available contained 50; the first two rows showed in 18 cigarettes a loss of only 7·6 per cent, which was not statistically significant (P = 0·2). The first two rows of another box (Table VI) were of more uniform composition, and showed, in 22 cigarettes, a significant loss of 13·7 per cent (P = 0·01). Three cigarettes in the 3rd row taken for ashing yielded exceptionally large amounts of arsenic

(mean of 3 = 63 μg./g.; this figure is 40 per cent above the mean of the other cigarettes ashed from this box), which caused the mean amounts in this row to be actually greater, by 5·4 per cent, in the ashed than in the unashed cigarettes.

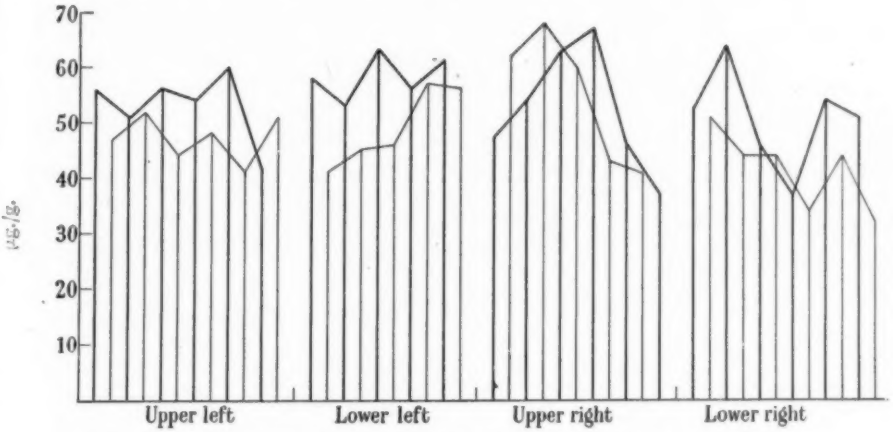

FIG. 2.—Consecutive analyses of cigarettes, and of ash and stump. Brand C.
——— Cigarette.
——— Ash and stump.

TABLE VI.—*Consecutive Analyses of Cigarettes, and of Ash and Stump. Brand C. Box of* 50. *Method of Thomas and Collier* (1945).

As_2O_3 μg./g. Cigarette.	As_2O_3 μg./g. Ash and stump.	Loss per cent.	P.	As_2O_3 μg./g. Cigarette.	As_2O_3 μg./g. Ash and stump.		Loss per cent.	P.
Upper left row.				Upper right row.				
56·4	36·6	..	..	47·1	61·7	mean	..	..
51·6	52·1	..	..	53·3	68·1	63·1	..	..
56·7	44·1	..	..	62·9	59·6		..	..
53·6	48·4	..	..	66·2	42·7		..	..
60·7	41·6	..	..	45·3	41·2		..	..
40·6	55·2	..	..	36·7	..		..	..
Mean:								
53·3	46·3	13·1	0·1	51·9	54·7		(+ 5·4)	0·7
Lower left row.				Lower right row.				
58·0	41·8	..	..	52·7	50·7		..	..
52·7	45·6	..	..	63·4	44·5		..	..
62·3	45·8	..	..	45·5	43·6		..	..
55·8	56·8	..	..	36·7	33·2		..	..
60·3	55·6	..	..	53·9	44·3		..	..
..	..	..	..	50·9	31·5		..	..
Mean:								
55·3	49·1	15·0	0·1	50·5	41·3		18·2	0·05
Both left rows.				Whole box.				
55·3	47·7	13·7	0·01	53·2	47·5		10·7	0·01

The 4th row showed a loss of 18·2 per cent, which is just significant (P = 0·05), in spite of the small numbers (12 cigarettes) involved. The analyses upon the whole box showed a significant loss of 10·7 per cent (P = 0·01). The whole course of the analyses on this box is shown in Fig. 2.

Several estimations on from 10 to 20 cigarette papers, whether bought in packets or taken from cigarettes, gave amounts between 0·75 and 2 μg. ; hence the quantity in a single cigarette is negligible. The papers and cork-tips from 10 C cigarettes gave 1·25 μg.

BRANDS OTHER THAN ENGLISH

Cigarettes of thirteen other brands were examined, some by the Gutzeit method and some by that of Thomas and Collier (1945). Three well-known American brands gave figures (25 to 47 μg./g.) rather lower than the means (50 to 55 μg./g.) of the English brands P and C (Table VII). All the eight Turkish brands showed

TABLE VII.—*Brands other than English.*

Brand.	Number of cigarettes.	As_2O_3 μg. per g.
American :		
Brand A	1	35·2
„ F	2	41·0, 47·0
„ S	5	30·0, 25·3, 26·3, 26·9, 31·2
Turkish :		
Brand R, Box 1	2	*Nil** ; (ash + stump) 1·4
„ R, Box 2	5	1·03 ; *nil* ; 4·3 ; *nil* (ash + stump) *nil*.
„ A	1 in two portions	0·5, 1·9
	4 whole	1·08, 1·08, 1·0, 0·9
„ G	1 in two portions	4·0, 2·1
	2 whole	1·1, 1·3
„ S	3	1·0, 1·0, 0·5
„ B	2	0·5, 0·5
„ C	3	*Nil**
„ P	3	3·4, 0·5, 1·2
„ M	4	3·3, 3·1, 1·7, 0·7
French	2	1·5, 0·5
? Rhodesian	3	4·1, 1·8, 2·6

* *i.e.* colour not distinctly different from that of blank.

very low amounts (*nil* to 4 μg./g.), and similar results were given by a packet brought from France and by cigarettes supposed to contain Rhodesian tobacco. After these results were obtained we came upon a short note by H. Popp (1928) of Frankfurt a. M. giving the following results :

	Arsenic p.p.m.
Tobacco from the Palatinate	5·1
„ „ Macedonia (cigarette tobacco)	0·7
„ „ Java	0·33
„ „ Brazil	4·6

He comments upon the much higher figures of Remington (1927), and suggests the possible importance of insecticides.

The possibility was considered that the very low results given by Turkish cigarettes were due to some constituent having an inhibiting effect on the re-

actions. Two portions weighing (*a*) 0·25 and (*b*) 0·24 g. were cut from a Turkish cigarette, and to (*b*) was added before combustion 0·8 ml. of the standard solution, containing 8 μg. As_2O_3. The Gutzeit method gave in (*a*) an amount not different from that of the blank, and in (*b*) 7 μg. As_2O_3.

DISCUSSION.

The most likely source of the arsenic present in some tobaccos, which has been suggested by several authors, is the spraying of the adult plants with insecticides, and this particulate method of application would explain the difficulty in obtaining concordant analyses. If the arsenic were absorbed by the roots one might expect a more uniform distribution. In some areas the seedlings only are sprayed, and presumably this arsenic is lost in the course of transplantation and further growth. We are obtaining data about the use of arsenical solutions in the areas where the tobaccos examined are grown.

Of the arsenic volatilized in smoking, a part must escape while the cigarette is not in the smoker's mouth, and a part of what he inspires is expired again, hence the amount retained is no doubt very small. We have no data about the size of the particles in which the arsenic is carried. The problem differs from those of other inhaled substances in that the nasal cavity, which is especially adapted to arrest particles, is not involved in inspiration. The percentages of arsenic volatilized in these experiments (7·6 to 18·2) are similar to those recorded, for cigarettes, by Thomas and Collier (1945) (mean 14 and 19), lower than those of Gross and Nelson (1934) (mean 23·3), and much lower than that of Remington (1927) (50). If a person smokes 50 cigarettes with a mean arsenic content of 50μg. and 15 per cent of this escapes, he has volatilized 0·375 mg. As_2O_3, which is the amount contained in 0·0375 c.c. of Fowler's solution (official dose 0·125 to 0·5 c.c.).

SUMMARY.

The arsenic content of 15 brands of cigarettes has been estimated and a range from *nil* to 106 μg./g. As_2O_3 has been observed ; the lowest amounts were found in nine brands classed as Turkish, French, and Rhodesian. The concentration of arsenic in cigarettes containing the larger amounts is very variable ; this irregularity is perhaps due to a method by which the arsenic may have been introduced, namely, by the spraying of the plants with insecticides. An estimate was made of the amount of arsenic volatilized in smoking, by comparing the content of cigarettes, and of the ash and stumps derived from them, but the wide range of arsenic content makes it difficult to infer the amount present originally in a cigarette which is to be smoked. The results indicated that from 7·6 to 18·2 per cent of the arsenic present was lost in smoking ; this figure is of the same order as but somewhat lower than that obtained by some previous investigators who have used artificial methods of smoking.

We wish to express our thanks to the British Empire Cancer Campaign, the Anna Fuller Fund and the Jane Coffin Childs Fund for Medical Research for grants. We are much indebted to Mr. Frank Goulden, A.R.I.C., for advice upon the estimation of arsenic.

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THE MECHANISM OF THE LIVER CATALASE DEPRESSING ACTION OF TUMOURS IN MICE.

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It now seems well established that tumours are capable of producing general systemic effects in the animal body. For example, Greenstein and his associates have shown that the activity of certain liver enzyme systems is affected to a considerable extent by the presence of a distant tumour, and of those investigated, liver catalase appeared to be the most sensitive. In fact a marked decrease in the activity of this enzyme has been observed in rats and mice bearing a variety of spontaneous, transplanted, and chemically induced tumours (Greenstein and Andervont, 1942). The evidence connecting the liver catalase depression with the presence of a tumour has recently been reviewed, both by Greenstein (1947) and by Weil-Malherbe and Schade (1948).

The precise mechanism by which this depression occurs is at present obscure. As Greenstein (1947) points out, there are two main processes which can account for the observed results. Either the tumour may release some toxic material into the circulation, or its excessive nutritive demands may abstract from the circulation some substance essential for the maintenance of a normal liver catalase level. With regard to the first alternative, most tumours contain varying proportions of necrotic tissue, and normal tissue may also be destroyed by tumour invasion. Consequently, quantities of tissue breakdown products are likely to be released into the circulation, and any toxic material associated with tumour growth may be due to such relatively non-specific processes. There is in fact evidence (Winzler and Burk, 1944) that abnormally large quantities of circulating protein breakdown products such as proteoses and polypeptides are found in tumour-bearing animals, and these authors did tentatively suggest that liver catalase depression might be due to this cause.

With regard to the second alternative, i.e. excessive nutritive demands of the tumour, Greenstein and Andervont (1943) showed that the depression was not simply due to the presence of growing tissue within the host, since catalase activity remained normal both in pregnant mice, and in mice bearing progressively growing, subcutaneously implanted, embryonic tissue mash.

Greenstein (1943) looked for a direct inhibitor of catalase in tumours, and in the livers of tumour-bearing animals, but the results were all negative. The kinetic behaviour of the enzyme in the livers of normal and tumour-bearing animals was also identical. Greenstein interpreted his experiments as indicating that the affected livers simply contained less catalase than normal, rather than the same amount of catalase plus an inhibitor, and put forward the interesting suggestion that the decrease in activity was due to an interference with the synthesis of the enzyme. However, this suggestion throws no light on the

manner in which the tumour exerts its effect, since the alternatives discussed earlier might all equally result in an interference with synthesis.

Weil-Malherbe and Schade (1948) made a careful study of the effect of injecting protein, and split protein products, into normal rats. However, no depression of liver catalase activity was observed even after long periods of administration of commercial peptone or sheep serum. Sheep serum in fact resulted in a rise in activity, the significance of which is unknown. Further, these authors investigated the possibility that the depression might be due to the exaggerated protein requirements of the tumour, by maintaining two series of tumour-bearing rats on high and low protein diets respectively, but no significant differential effect on the course of the process was observed.

Most previous work appears to have been carried out with relatively massive tumours (roughly within the range of 5 per cent to 50 per cent of body weight). The nutritional status of the animals can hardly be normal under these conditions, which may cast doubt on the validity of any interpretation based on the results. It is true, however, that while Miller (1947) found that starvation up to about 7 days caused a marked depression in liver catalase level, he also showed that total liver protein fell in an approximately parallel manner. Reference of catalase level to total protein as a standard, therefore, will minimize non-specific effects due to simple inanition.

It would seem of some interest to attempt to gain a fuller understanding of the relationship between tumours and the liver catalase depression. There appears to be a possibility that the effect is of a relatively specific nature, given only by malignant tissue, but the evidence presented in the literature does not enable a decision to be made on this question.

In this paper a study has been commenced of the early effects on liver catalase activity resulting from the injection of tumour tissue. So far as the results go they are in support of the supposition that tumours contain a substance not present, or present in much lower concentration, in normal tissue, which is capable of depressing catalase activity.

MATERIALS AND METHODS.

Animals.

Young adult mice (weight approximately 25 g.) of two strains have been employed in this investigation: (1) the FF fawn strain (Glaxo Laboratories, Ltd.), and (2) the Schofield albino strain (supplied by S. Schofield & Co., Oldham). Neither of these are a pure line, although the FF's have been bred by litter-mating for 3 years. During the period of the experiment the diet of the animals consisted of "rat cubes" (North-Eastern Agricultural Co-operative Society, Aberdeen) and water *ad libitum*. As a routine the mice were starved for 12 hours before catalase determination.

Tumours.

Most of the work has been carried out with the transplanted tumour Sarcoma 37 (obtained from the Chester Beatty Research Institute), which grows rapidly in both strains of mice after subcutaneous inoculation. This method of administration has been used throughout. Carcinoma 63 (from the Imperial Cancer Research Fund laboratories) has also been employed, but this tumour grew much

less rapidly, and failure to "take" has not been uncommon. Only actively growing tissue has been used in the experiments except where otherwise stated, any necrotic areas being discarded.

Preparation of enzyme.

Animals were killed by decapitation, blood allowed to drain from the carcass, and the liver rapidly dissected out. After removal of the gall bladder the liver was weighed approximately, and homogenized in ice-cold glass-distilled water, using a Ten Broeck grinder. The resulting homogenate was made up to a volume corresponding to 10 ml./g. liver, and a part diluted 10 times, again with ice-cold glass-distilled water. For the determination of catalase activity, 0·2 to 0·4 ml. of dilute suspension were taken, and 0·5 ml. of the concentrated suspension for nitrogen estimation. As has been pointed out in the introduction, it is desirable to refer the catalase activity to total liver protein as a standard. Since, however, the non-protein nitrogen amounts to less than 10 per cent of the protein nitrogen and appears relatively constant, total nitrogen has been employed as a standard in this investigation. This has been estimated by a semi-micro Kjeldahl method.

Liver catalase estimation.

Catalase has been estimated by measuring the hydrogen peroxide remaining in a given volume of standard solution after allowing the enzyme to act for a given time. Since only relative catalase activities are of interest in the present work, it was decided not to employ the tedious method of Kat. f measurement, but to construct a standard curve of enzyme concentration in arbitrary units (using different volumes of the same liver homogenate) against hydrogen peroxide decomposed in 4 minutes. This curve could then be used to measure enzyme concentration in subsequent experiments. Since the rate of hydrogen peroxide decomposition falls off rapidly with increasing enzyme concentration, it was found necessary to construct a curve covering a relatively wide range of concentrations. The technique employed for this and for subsequent estimations has been as follows: Hanging buckets containing the appropriate amount of enzyme were dropped into 25 ml. of M/40 hydrogen peroxide (A. R. quality) in M/50 phosphate buffer, pH 6·8, and the reaction stopped after 4 minutes by the addition of 3 ml. of 50 per cent sulphuric acid. The reaction was allowed to occur in 100 ml. conical flasks maintained at 0°C in an ice bath. Five ml. of 15 per cent W/V potassium iodide and 3 drops of 5 per cent W/V ammonium molybdate were then added, and the liberated iodine titrated with N/20 sodium thiosulphate. Blank determinations were made using flasks in which sulphuric acid was present from the beginning. The quantity of hydrogen peroxide decomposed by the enzyme is then given by the difference between the two results. All enzyme determinations have been made in duplicate, and, for routine estimations, the quantity of enzyme taken has normally decomposed between 1/3 and 2/3 of the substrate during the course of the reaction. The standard curve of enzyme concentration against H_2O_2 decomposed was constructed by combining the results of two experiments on the same liver homogenate. As expected, the slope decreased steadily with increasing enzyme concentration, but when the results are plotted as reciprocals (Fig. 1) they lie on a straight line, and the line of nearest fit has been calculated by standard statistical procedure. In routine

determinations, catalase activity in arbitrary units has been calculated by substituting the quantity of hydrogen peroxide decomposed in the equation to this line, and the result divided by the nitrogen content in mg. of the amount of enzyme solution used, the final result therefore being expressed in arbitrary units/mg. N.

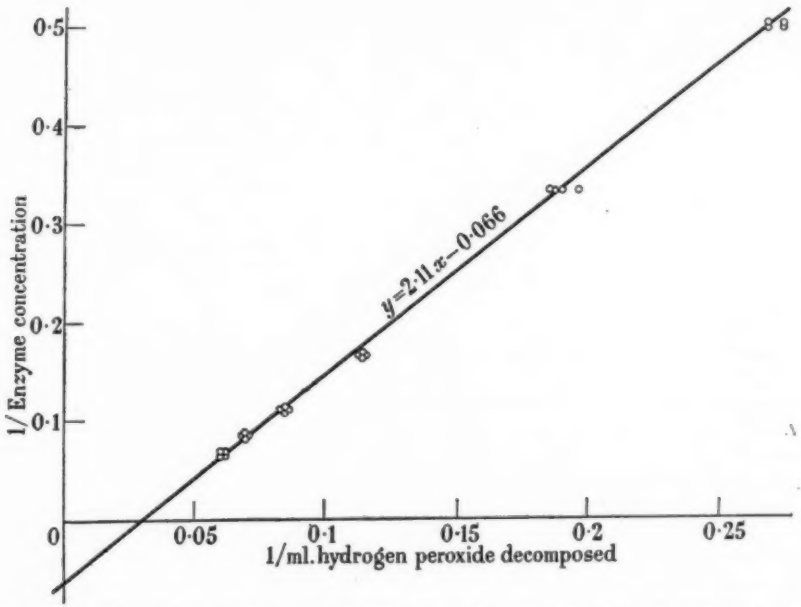


FIG. 1.—Reciprocal of catalase concentration in arbitrary units against reciprocal of hydrogen peroxide destroyed in 4 minutes. The straight line is the regression of ordinate on abscissa.

RESULTS.

A large group of equal numbers of male and female FF mice were each injected subcutaneously with 0·3 ml. of a coarse homogenate of S37 tissue equivalent to 50 mg. of original tumour. This was obtained by dissecting out tumours previously inoculated into FF mice, removing any necrotic areas, and homogenizing the actively growing tissue in a loose-fitting Ten Broeck grinder just sufficiently to give a coarse suspension. Fig. 2 shows the variation in liver catalase activity with time after this procedure. The extreme left-hand group of 16 animals (at time T = 0) are the control group. Groups of 3 male and 3 female mice were killed at intervals up to 14 days after the injection of the tumour homogenate, and their liver catalase activity estimated. In Fig. 2 the crosses represent the arithmetic mean values of the catalase level of the groups. It may be seen that at 24 and 48 hours after injection the level is considerably depressed, that it rises approximately to normal at 4 days, and then falls progressively until 14 days, when the experiment was terminated. There are several points

of interest regarding this graph. Firstly, palpable tumours did not make their appearance until 4 to 6 days after the injection, subsequently growing steadily. Because af this, and also since the amount of the initial (24- and 48-hour) depression is not approached again until the new tumours are of a considerable size it seems not unreasonable to suggest that the initial drop is due to some material contained in the injected tumour homogenate, that this effect is rapidly exhausted (4 days) and that only the second depression (> 4 days) is due to the presence of the new tumour. The graph also shows that there is a sex difference in the normal control catalase level, which is higher in the males than in the females : the male

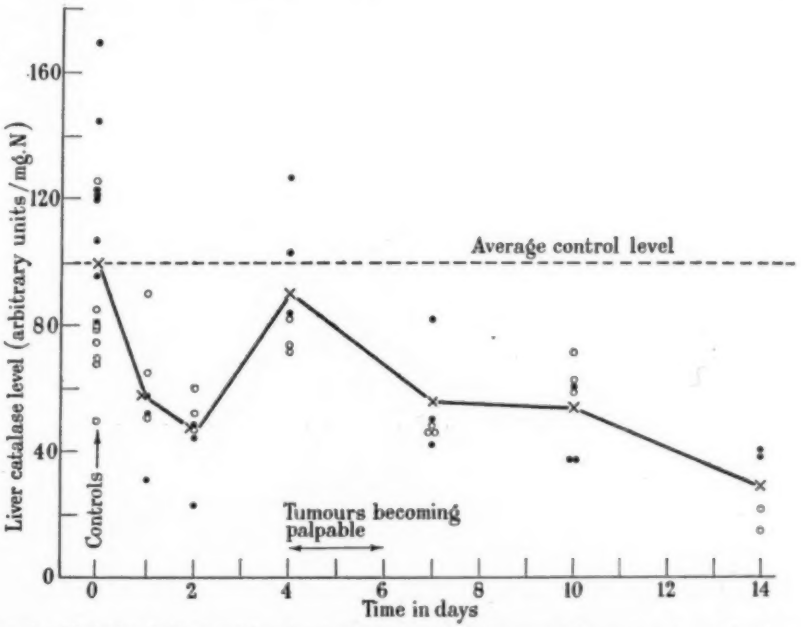


FIG. 2.—Variation in liver catalase level after injection of Sarcoma 37 homogenate. FF mice. Open circles—females ; black circles—males. The crosses represent the arithmetic mean values of the group levels.

average is 120 units, and the female 80 units. Although the treated groups become very small when split up, the initial (24- and 48-hour) percentage depression in catalase activity appears to be considerably greater in the males. The experiment was repeated with Schofield albinos, using S37 taken from the same strain, and larger treated groups to enable the sex difference in initial effect to be examined further. The results are given in Fig. 3, where it will be seen that there is an early depression in enzyme level, a rise approximately to normal in 4 days, and a subsequent progressive fall. As before, the controls are the extreme left-hand groups, and the crosses represent arithmetic mean levels. Once again there is a sex difference in normal catalase level (males 153 units, females 123). The greater sensitivity of the males to the early effect is apparent, despite the fact

that the mice were given the same quantity of material irrespective of sex, the females, being appreciably lighter, therefore receiving a higher dose/body weight. As in the FF mice, the growing tumours were not palpable before about 5 days after injection. In this experiment measurements of tumour weight were also made, and the results appear in Table I. Table I shows that up to a tumour

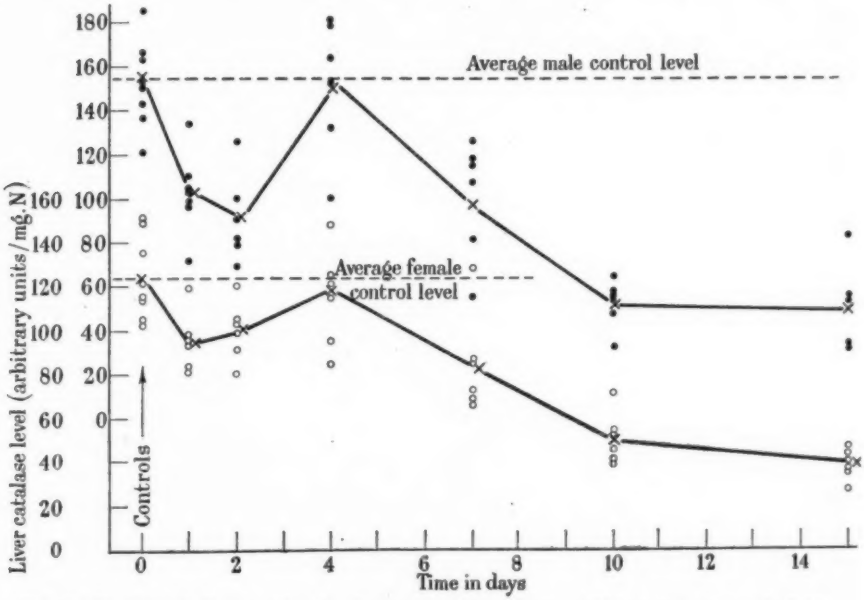


FIG. 3.—Variation in liver catalase level after injection of Sarcoma 37 homogenate. Schofield mice. Open circles and outer ordinate scale—females; black circles and inner ordinate scale—males. The crosses represent arithmetic means.

weight of about 1·5 g. the tumour grew more rapidly in the males. Above this weight the tumours became considerably necrotic, and the difference in growth rate was less marked. Greenstein (1943) has already stated that tumours exert no selective effect as far as sex is concerned on liver catalase. This appears confirmed by the collected results from several experiments given in Fig. 4, which is a plot of S37 tumour weight in grammes against percentage depression in catalase level. Each point represents 6 tumour-bearing animals, and has been obtained from the arithmetic mean of the catalase level for the treated animals, and the arithmetic mean of the appropriate control group. The tumour weights (obtained by dissecting out and weighing the tumours) are also averaged for each group. This method of presenting the experimental data is by no means ideal, but the individual results would be very difficult to obtain, since no information is available regarding the original catalase level of a tumour-bearing animal. The graph (Fig. 4) indicates that for a given tumour *weight* there is, at least, no marked sex difference in catalase depression, and also that the depression is asymptotic to a level just below 70 per cent.

TABLE I.—*Schofield Albino Mice : Sex Difference in S37 Tumour Growth Rate.*

Each group contains six animals.
Extensive necrosis was present at 14 days in both sexes.

	Time after inoculation of tumour homogenate (days).	Tumour weight. Mean ± standard deviation (g.).
Males	4	—
	7	0·50 ± 0·15
	10	1·55 ± 0·58
	14	3·0 ± 1·0
Females	4	—
	7	0·28 ± 0·12
	10	0·85 ± 0·31
	14	2·4 ± 0·7

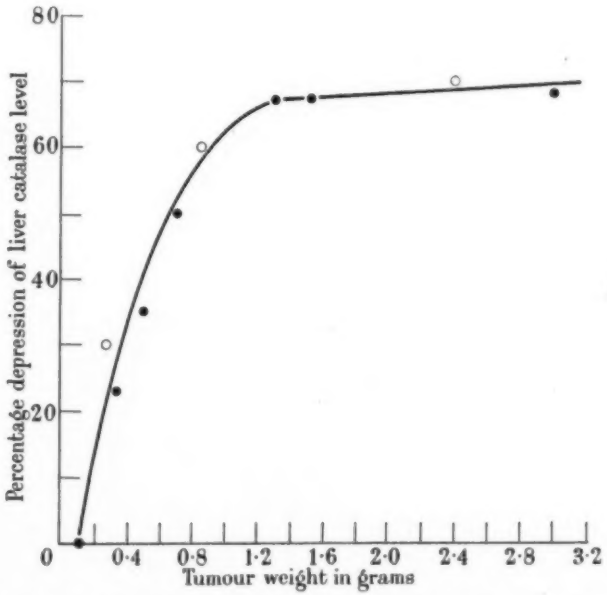


FIG. 4.—Dependence of catalase level upon tumour weight. Open circles—females ; black circles—males. Each point represents the average of six tumour-bearing animals.

Greenstein and Andervont (1942) have also followed the time course of the catalase depression in dilute brown mice grafted with the same (S37) tumour. Fig. 5 shows a graph constructed from a table given in their paper. It is obvious that there is a considerable difference between their results and those obtained in this laboratory, although there are points of similarity. According to the graph, these authors obtained a rapid fall in 2 days, after which the level remained approximately constant until 4 days, and then fell once more. The salient point is that their results cannot be separated into " early " and " late " effects in the absence of the 4-day rise to normal. Since this difference between their

results and those obtained here is of much importance it was decided to attempt to find out how it arose. Their tumour must have grown extremely rapidly since they obtained a 90 per cent depression in 7 days, and Greenstein and Andervont (1942) probably employed the usual technique of grafting small pieces of tumour subcutaneously rather than injecting a homogenate. In order to see whether the difference in results could be explained on this basis a group of

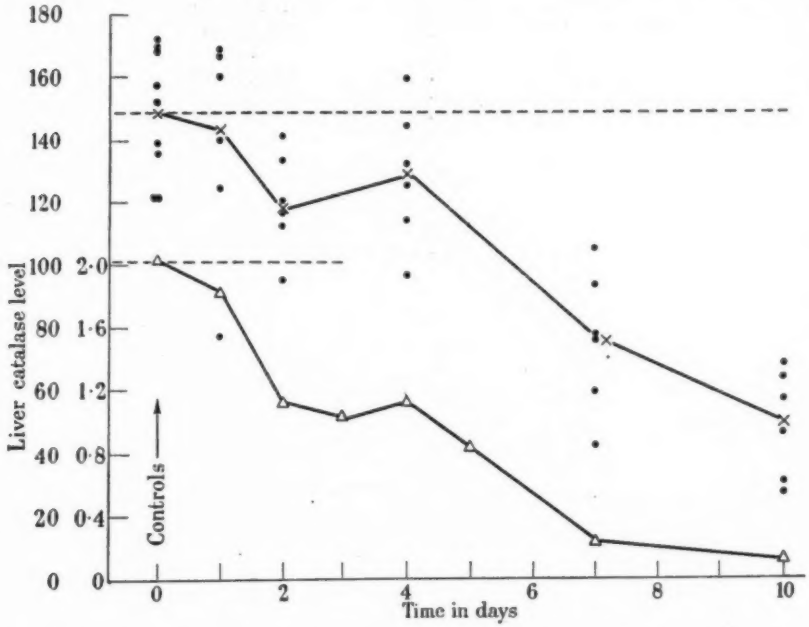


FIG. 5.—Upper graph (outer ordinate scale): Variation in liver catalase level after implantation of pieces of Sarcoma 37. Schofield male mice. Lower graph (inner ordinate scale): Variation in catalase level after S37 implantation, constructed from data of Greenstein and Andervont (1942). Dilute brown mice.

Schofield male albinos was inoculated with 50 mg. each of S37 in relatively large pieces, using a wide bore trocar and cannula. The results are also presented in Fig. 5, and the similarity with those of Greenstein and Andervont (1942) is apparent. As might be expected, compared with those experiments in which the tissue was coarsely homogenized, a more rapid tumour growth occured. It is fairly obvious, from the results already given, that if the onset of tumour growth is sufficiently rapid to affect the catalase level at 4 days, the rise to normal after the initial drop will be obscured, or perhaps entirely obliterated. Fine homogenization in a Ten Broeck grinder was found to delay tumour growth still further. Mice were injected with 50 mg. in 0·3 ml. of tumour tissue treated in this manner, and as shown in Fig. 6, the catalase level after an initial depression was almost normal at 4 days, and remained normal until 7 days. Palpable

tumours did not appear on this occasion until 6 to 7 days after injection, and the average tumour weight at 7 days was 100 mg. It appears, therefore, that tumours of this small size are without significant effect on the catalase level. As the graph shows, the level had fallen at 10 days, by which time the tumours were considerably larger (300 to 400 mg. aproximately).

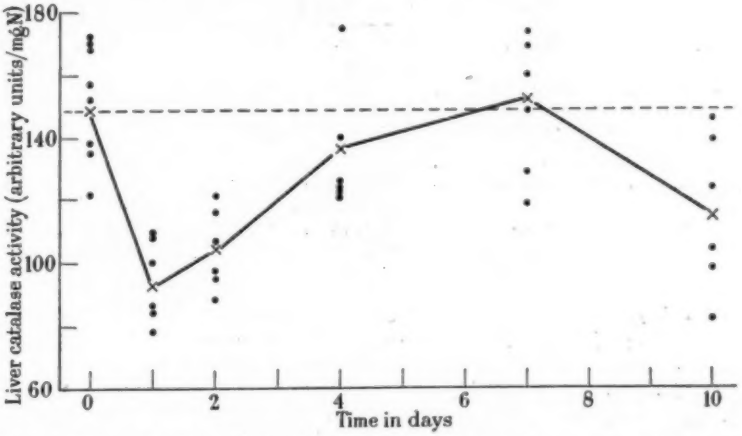


FIG. 6.—Effect of fine homogenization (S37 tumour) on the time course of the catalase depression. Schofield male mice.

A number of normal tissues have been coarsely homogenized, and injected subcutaneously into male mice, which, as has been already pointed out, are more sensitive than females. The tissues investigated include whole embryo, which was obtained from a 10 to 14 days pregnant normal Schofield female. Since the initial (24 and 48 hour) depression is of primary interest, the effects on catalase level have been followed over a period of 4 days, the results appearing in Table II. None of the observed alterations in level at 24 hours, 48 hours, and 4 days after the injection of normal tissue are statistically significant. In two of the experiments, however, with mouse spleen and rat thymus the control level was rather low. Appreciable variations in normal level from batch to batch both with Schofields and FF's are in fact, not unusual. Table II also gives the results of an experiment using coarsely homogenized Carcinoma 63. As has been previously pointed out, this tumour does not grow very satisfactorily after subcutaneous inoculation in the strains of mice used. In this experiment only about half the mice developed tumours, and then not until 10 to 14 days after treatment. The results in Table II show that a 24- and 48-hour depression was observed, although an appreciably smaller one than with S37, and that the catalase level rose to normal at 4 days. As with S37, the males were more sensitive, the depression in females being only on the border line of significance. Because of the much delayed onset of tumour growth, and because "takes" only occurred in about half the animals it is not possible to state definitely whether the females showed a greater resistance to tumour growth, although there appeared to be a tendency in this direction.

TABLE II.—*Effect on Liver Catalase of the Subcutaneous Injection of Coarsely Homogenized Tissues.*

Each treated group contains six animals (seven in the whole embryo experiment) and each control group eight.
Results are given as arithmetic means ± standard deviations.

Strain.	Tissue.	Catalase level in arbitrary units/mg.N. Controls.	24 hours.	48 hours.	4 days.
		Normal tissues.			
FF males	FF muscle	112 ± 23	105 ± 21	125 ± 22	113 ± 33
Schofield males	Schofield spleen	104 ± 14	101 ± 16	108 ± 26	116 ± 14
,, ,,	Albino rat thymus	104 ± 14	113 ± 14	113 ± 17	114 ± 19
,, ,,	Schofield whole embryo	132 ± 28	123 ± 22	123 ± 29	128 ± 17
,, ,,	Schofield brain	140 ± 25	131 ± 34	127 ± 19	131 ± 27
		Malignant tissue.			
,, ,,	Carcinoma 63	125 ± 14	101 ± 13	95 ± 13	125 ± 20
Schofield females	,,	90 ± 14	75 ± 19	79 ± 13	95 ± 11
Schofield males	Necrotic S37 tissue	140 ± 25	145 ± 28	92 ± 33	117 ± 6

An experiment with coarsely homogenized necrotic S37 tissue yielded the following result: No significant depression was observed at 24 hours, but by 48 hours the level was considerably below normal, beginning to rise at 4 days. The results are given in Table II.

Since attention does not appear to have been drawn to a sex difference in normal mouse catalase levels, some results for males and females drawn from the same batches are given in Table III.

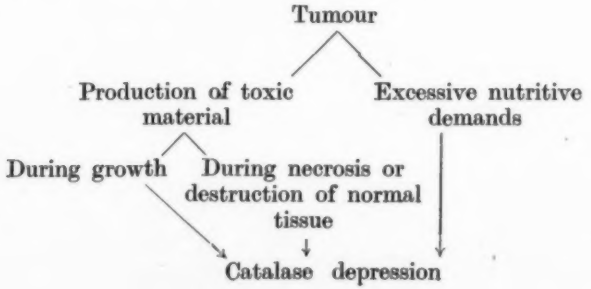
TABLE III.—*Sex Difference in Catalase Level of Normal Mice.*

Each group consists of eight animals.
Results are given as arithmetic means ± standard deviations.

Batch.	Strain.	Male level.	Female level.
1	FF	120 ± 26	80 ± 21
2	Schofield	153 ± 16	123 ± 18
3	,,	125 ± 14	90 ± 14

DISCUSSION.

As pointed out in the introduction, the alternative mechanisms by which tumours may cause a catalase depression can be given thus:



The results already given show that Sarcoma 37, administered in an appropriate manner, is capable of producing a highly significant depression in liver catalase level before any active growth is observable, and the degree of this initial depression is not again reached until a tumour of appreciable size has grown. It is hardly possible that this early depression can be a result of any nutritive demands on the organism, and can only be due to some substance or substances present in the injected tumour tissue. Assuming that the early and late depressions are not entirely unconnected, it is suggested that the tumour is exerting its effect on catalase by continuously releasing this material into the circulation. Since 50 mg. of homogenized tumour tissue (administered by the relatively inefficient method of subcutaneous injection) is sufficiently active to depress the catalase level very considerably for approximately 2 days, it is probable that only a comparatively small continuous production would be required to account for the observed results. Fig. 4 indicates that the results cannot be explained, at any rate in this case, on the supposition that tumour necrosis is responsible. In the strains of mice used, practically no necrosis is visible in S37 tumours until a weight of 1 to 1·5 g. is reached. As Fig. 4 shows, considerable depressions in activity (up to at least 50 per cent) are observed with tumours below this weight containing little or no necrosis. Further, the point at which the graph flattens corresponds approximately to the tumour weight at which necrosis commences, and, for example, a tumour weighing 2 to 3 g. which is roughly half necrotic produces only about the same percentage depression as a much smaller tumour containing no necrosis. Also, after subcutaneous injection, these tumours grow in the subcutaneous space and do not, as a rule, perforate the abdominal wall until they weigh 4 to 5 g. at least. Consequently little or no normal tissue can be destroyed by the growth of the tumour ; and even if this process were occurring to some small extent, the flattening of the curve at a given point would be unexplained. The interpretation of the result using injected necrotic tissue is not easy. The catalase depressing substance appears still to be present, since a marked fall in activity was observed at 48 hours. The normal level at 24 hours might be explained on the supposition that this substance had become bound in some way and was slowly released after injection. However, the results given in Fig. 4 strongly suggest that the most important factor is the release of some material by the activity growing areas of the tumour. Whether this acts by interfering with the synthesis of the enzyme is a matter for conjecture at present.

Little reference has been made in the literature to a sex difference in tissue catalase. Schultz and Kuiken (1941) briefly mention that normal male rats have higher liver and kidney levels than females, and Serfaty (1946), working with the erythrocytes of fowls, found the level to be higher in cocks than in hens. It seems reasonable to suppose that the maintenance of a normal catalase level is to some extent under hormonal control, and the different initial response of males and females to tumour tissue may indicate that the primary effect is not on the liver. Experiments with castrated or adrenalectomized animals are an obvious first step in the further investigation of this aspect.

The importance of homogenizing the tumour tissue is apparent from the results given in Fig. 3, 4, and 5. These graphs make it clear that not only does homogenization enhance the initial depression (as might be expected if the effect is due to some substance contained in the tumour cells) but that such a procedure

delays the new tumours, thus enabling the catalase level to rise to normal before there is sufficient tumour growth to exert any effect. They also give a reasonable explanation of the results obtained by Greenstein and Andervont (1942) in which no rise to normal appeared. Their results show only a progressive fall in catalase level after the tumour implantation, and provide no evidence which could suggest that tumour tissue contains a toxic product capable of depressing liver catalsea activity.

No significant alterations in catalase level were shown to follow the injection of normal tissue. In some experiments the arithmetic mean of the results for the treated animals have been slightly lower than the arithmetic mean values for the controls, in others, slightly higher. The variations on the individual results are such that variations of $\pm$ 10 per cent in the arithmetic mean values are certainly not significant. It is impossible at present, therefore, to say whether normal tissues are capable of causing a slight depression, or whether the effect is wholly specific to tumours. Obviously it is important at the present stage to attempt some fractionation of the active principle. After this process the material could be compared with similar fractions from normal tissue, and a sufficiently large dose given to show whether the catalase depressing material is present at all in normal tissue. The nature of the material is of course quite unknown at present.

The indication that there may be a parallelism between the initial catalase depression and the resistance of the animal to the tumour is also of interest. It is already known (Greenstein and Andervont, 1942) that, in general, the greatest falls in liver catalase level are produced by rapidly growing tumours, and, in fact, certain slow-growing tumours produced little or no effect. In the present investigation it appears that S37 tissue, which grows readily, gave a greater 24 and 48-hour depression than Carcinoma 63, which does not. Further, the females, which showed smaller depression than the males, appear more resistant to tumour growth.

Extension of the work to other tumours would seem most desirable at this point, and it is proposed to continue along these lines.

Since this paper was submitted for publication my attention has been drawn to a paper by Nakahara and Fukuoka (1949). These authors have observed depressions in the mouse liver catalase following the injection of alcohol precipitated fractions from a number of human tumour tissues. Other preparations did not inhibit liver catalase *in vitro*.

SUMMARY.

Following the subcutaneous injection of homogenized Sarcoma 37 tissue into two strains of mice, the liver catalase activity fell significantly at 24 and 48 hours, subsequently rose to normal by the 4th day, and diminished again during the growth of the new tumours. Carcinoma 63 also gives an initial depression.

Injection of a variety of normal tissues, including whole embryo tissue, produced no significant alteration of liver catalase level.

The results are interpreted as providing evidence that tumours exert their action on liver catalase by releasing some toxic product into the circulation.

The evidence also suggests that necrotic processes are not responsible, but that the effect is primarily associated with actively growing tissue.

A significant sex difference in normal liver catalase level was found. There was also a sex difference in the initial response to the injection of tumour material, the males, which have the higher normal level, being more sensitive.

My thanks are due to Dr. M. H. Salaman for his advice and encouragement, and to Professor S. P. Bedson for his interest. I am also indebted to Mr. L. J. Hale and Mr. M. Ridler for skilled technical assistance.

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THE INDUCTION OF TUMOURS FOLLOWING THE DIRECT IMPLANTATION OF 20-METHYLCHOLANTHRENE INTO THE UTERUS OF MICE.

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In 1944 Castellano and D'Amour described uterine tumours in rats following the introduction of 10 mg. of methycholanthrene in paraffin wax into the uterine horn. Simultaneous administration of oestrogen improved the yield, whereas progestin (? progesterone) had a slight inhibitory effect. Greenblatt and Kupperman (1947), also using the rat, observed squamous metaplasia of the horn and a doubtful precancerous lesion in the cervix uteri, but no tumours, when a similar dose of methylcholanthrene was introduced. Pan and Gardner (1948) reported the development of numerous carcinomas and sarcomas in mice of several strains, when subcutaneous implants of cervical and uterine epithelium were made together with small crystals of methylcholanthrene.

In the experiments described here uterine tumours were induced by the introduction of methylcholanthrene directly into the uterine horn in two types of mice and the effects of various hormonal factors were studied.

METHODS.

The experiments were performed on two types of mice: inbred CBA mice, maintained by brother-sister mating in the Department of Cancer Research at Leeds, and white mice obtained from dealers.

Implantations were performed under ether anaesthesia. The following methods were used, and found satisfactory in animals weighing as little as 9 g.:

(1) The abdomen was opened and the viscera gently pushed aside by means of a swab. A fine silk suture was passed around the upper part of the left horn and fastened in such a way that the whole horn became readily accessible. The lower part of the horn was then ligated, and a small incision made with fine scissors into the upper part. In the earlier experiments a "gun," i.e. a fine glass tube with accurately fitting rod acting as piston, was charged with crystalline 20-methylcholanthrene (obtained from Light & Co., Ltd.). The gun was introduced through the incision, a fine silk ligature placed around it a short distance below the incision, and the carcinogen pushed out into the lumen of the horn. As the gun was withdrawn the ligature was tightened, the implant of carcinogen thus remaining between the two ligatures. In later experiments a hypodermic needle, without the shoulder, was used as the "gun," and the stilette used as piston. With this method it is easy to introduce the material even in very small uteri.

(2) The same method as (1) was used to introduce a 1 per cent solution of methylcholanthrene in lard (or 1 per cent methylcholanthrene and 1 per cent. oestradiol dipropionate in lard). A tuberculin syringe and blunt metal needle were used to introduce the material.

The weight of methylcholanthrene introduced as crystals by Method (1) was approximately 0·1 mg. The amount of material introduced in solution in lard varied considerably, depending on the size of the uterus, but there is no doubt that the weight of methylcholanthrene introduced with Method (2) was less, and sometimes considerably less, than that introduced into the uterus with Method (1).

(3) This method was similar to (1) and (2), except that the upper part of the horn through which the material was introduced was removed. This was done to prevent the development of sarcoma in connective or muscular tissue at the site of incision which might have become contaminated with small quantities of carcinogen.

(4) This method was devised in order to introduce material into the uterus without any incisions. The results obtained were not satisfactory, in that no malignant changes developed. The method is, however, described, as it may prove of value to other investigators.

The uterus was exposed, and the lower part of the right horn and upper part of the left horn ligated. A ligature was placed loosely around the lower part of the left horn. The upper part of the vagina was then exposed and dissected free from surrounding tissue and partly separated from the rectum. A stout ligature was loosely placed around the vagina just above the symphysis pubis. The ligature included the urethra (which can be separated if desired, but no harm appeared to be done by including it in the ligature). A solution of methylcholanthrene in lard (warmed to body temperature) was contained in a tuberculin syringe with a rather thick blunt-ended needle. The needle was introduced into the vagina and pushed up above the level of the ligature, which was then tied (with one knot) around the needle and held tied by an assistant. Pressure was then applied, rather carefully, to the piston of the syringe and the material forced through the cervix into the left horn of the uterus. That material, and not merely air, was actually introduced into the uterus was shown by using lard coloured red or blue with a suitable dye. The ligature around the lower end of the left horn was tied, the ligature around the vagina released and removed, and the needle with syringe withdrawn. The material remaining in the vagina was then thoroughly swabbed out.

With all the methods the abdomen was closed and, if ovariectomy had to be performed, this was done immediately afterwards by posterior incisions.

The abdominal organs were carefully palpated at regular intervals to determine the first appearance of uterine tumours, but the mice were not killed until they appeared to be ill or until there was no doubt about the presence of an abdominal tumour.

Mice receiving oestradiol dipropionate were smeared twice weekly, and a record kept of the duration of action of the oestrogen.

RESULTS.

Of 15 immature CBA mice, 12 developed tumours of the uterus, as follows: 4 had carcinomas, 6 sarcomas, and 2 both types of tumour (Table I). Of 94 white

mice, 20 developed tumours, 13 being carcinomas, 6 sarcomas, while one mouse had both types (Table II).

Most of the tumours attained a large size (e.g. 2 × 2·8 × 3·0 cm.), distorting the uterine horn and displacing other abdominal organs. The surface was usually smooth, but in the case of some of the carcinomas it was "craggy." On section, the tumours presented a homogeneous translucent appearance, sometimes with haemorrhagic and necrotic areas, except in the case of the squamous cancers,

TABLE I.—*Occurrence of Tumours in Immature CBA Mice.*

Spayed.							Intact.		
Crystals.			Lard solution.				Crystals.		
Mouse number.	Months.	Result.	Mouse number.	Months.	Result.		Mouse number.	Months.	Result.
108	8	S	106	5	S		97	3½	S
103	9	S + AC	107	8	S + AC		99	6	C Sq.
104	9	AC					96	10	S
105	11	S					93	10	S
							94	15½	AC
							98	16	AC Sq.
							100	17½	—
							101	17½	—
							95	17½	—
4		4	2		2		9		6

S = sarcoma; AC = adenocarcinoma; AC Sq. = adenocarcinoma with squamous metaplasia; C Sq. = squamous cancer.

which were usually granular and opaque on cut section. Ascites, either blood-stained or chylous, was frequent.

The sarcomas were locally invasive and grew into the voluntary muscle of the abdominal wall, in one case encircling breast ducts (Fig. 1) and in another a rib, and also into the pancreas (Fig. 2), the rectal wall (Fig. 3) and the ureter. No metastases were seen. The sarcomas were all spindle cell in type and appeared to arise in the uterine wall. All but one were regarded as fibrosarcomas (Fig. 1, 4); the one exception arose in CBA mouse 96 and was a leiomyosarcoma (Fig. 5). Several were pleomorphic, with multinucleated cells and bizarre nuclei (Fig. 4). When combined with carcinoma, the two types of tumour appeared to be of separate origin and not derived from spindle-cell anaplasia of an epithelial tumour. In several mice there was marked hyperplasia of the uterine glands, which lay surrounded by sarcoma tissue (Fig. 6, 7).

The carcinomas were classified as adenocarcinomas (Fig. 8), adenocarcinomas with squamous metaplasia (Fig. 9, 10, 11), or squamous carcinomas (Fig. 12). They varied in size from microscopic glandular formations to large invasive tumours. Cystic and glandular hyperplasia of the endometrium was not infrequently observed, either concomitantly with cancer or as a possible precursor of it. Direct invasion of the voluntary muscle of the abdominal wall, the diaphragm and the pancreas was seen. In white mouse 23 there was a metastasis in a regional

TABLE II.—*Occurrence of Tumours in White Mice.*

1. Spayed. Crystals. Mature.			2. Spayed. Crystals. Immature.			3. Spayed. Lard solution. Mature.			4. Spayed. Lard solution. Immature.		
No.	Ms.	Res.	No.	Ms.	Res.	No.	Ms.	Res.	No.	Ms.	Res.
50	6	—	35	6	—	75	11½	—	119	11	—
77	8	—	60	11	—	69	12	—	116	15	—
20	8	S	42	11½	—	70	13½	—	118	15½	—
27	8½	—	84	13½	—	74	18	—	122	15½	—
56	9½	—	36	14½	—	131*	4½	C Sq.	117	15½	—
28	10	—	86	17½	—	132*	4½	C Sq.	114	15½	—
37	11	AC Sq.	85	17½	AC	129*	7	—			
45	11	—	87	17½	—	130*	10	—			
51	11	—	83	17½	—	133*	10½	—			
23	12	AC Sq.	88	17½	—	137*	14	—			
21	13	AC	44	18	—	136*	14	—			
22	13	—	58	18½	—	125*	14½	—			
			67	18½	—	126*	14½	—			
			66	18½	—						
—	—		—	—		—	—		—	—	
12		4	14		1	13		2	6		0

5. Intact. Crystals. Mature.			6. Intact. Crystals. Immature.			7. Intact. Lard solution. Mature.			8. Intact. Lard solution. Immature.		
No.	Ms.	Res.	No.	Ms.	Res.	No.	Ms.	Res.	No.	Ms.	Res.
7	5	S	38	4	S	82	10	C Sq.	124	11	—
23	7	—	40	5	S, AC	79	13	AC	121	15½	—
14	8	—	123	6½	—	113	13	—	120	15½	—
1	9	—	90	8	AC	112	15	—			
18	10	S	65	8½	S	73	16	—			
3	10	—	32	10	—	81	16	—			
11	11	AC	30	11	—	111	16	—			
24	11	—	61	11½	—	110	16½	—			
17	12	—	33	12	—	80	18	—			
29	12	—	34	12	—						
68	12	—	62	13	—						
6	13	—	64	18½	—						
10	13	—	63	18½	—						
19	13	—									
25	13	—									
39	13½	—									
15	15	—									
9	15	AC									
8	16½	S									
16	17	AC									
13	18	—									
5	19	—									
26	20	AC									
12	20½	—									
—		—	—		—	—		—	—		—
24		7	13		4	9		2	3		0

* These 9 mice received 1 per cent oestradiol in lard at the time of introduction of the methylcholanthrene.

Abbreviations as in Table I.

lymph gland (Fig. 13). Transplantation of the tumours was not attempted, as their malignant nature was not in question.

DISCUSSION.

The object of the experiment was to find out whether the direct introduction of a carcinogen into the uterine horn would be effective in inducing carcinoma of the endometrium in mice, and if so, whether the incidence of tumours could be influenced by hormonal factors, or by differences in technique. In 109 mice 20 endometrial carcinomas were induced, an incidence of 18·3 per cent. In addition, 14 sarcomas were observed, an incidence of 12·8 per cent.

Effect of strain.

A higher incidence of both carcinoma and sarcoma occurred in CBA than in laboratory white mice (Tables I, II), in spite of the fact that all the CBA mice were immature at the time of implantation. The difference in carcinoma incidence (CBA 40 per cent, white 14·9 per cent $\pm$ 13·4) is nearly but not quite statistically significant (Table III); that in sarcoma incidence (CBA 53·3 per cent, white 7·4 per cent $\pm$ 13·4) exceeds the conventional level of statistical significance. But when immature CBA mice are compared with immature white mice (Table III), then the difference in carcinoma incidence becomes significant (CBA 40 per cent, white 8·3 per cent $\pm$ 13·4). The date of appearance of carcinomas was also earlier in CBA mice than in white mice (average 10·5 months in CBA mice

EXPLANATION OF PLATES.

FIG. 1.—CBA mouse 97, treated for 3½ months. Invasion of the subcutaneous tissue of the abdominal wall by spindle cell sarcoma. On the left the inguinal lymph gland; below, a breast duct and adipose tissue. × 100.

FIG. 2.—CBA mouse 106, treated for 5 months. Invasion and destruction of pancreatic issue by spindle cell sarcoma. × 100.

FIG. 3.—Another field from the same tumour as in Fig. 2, showing invasion of the rectal wall by sarcoma. Above and to the right are the rectal glands and submucosa invaded by loose sarcoma tissue; passing obliquely across the figure is the muscle of the rectal wall; below is the main mass of sarcoma. × 95.

FIG. 4.—White mouse 20, treated for 8 months. Pleomorphic spindle cell sarcoma, with giant cells. × 100.

FIG. 5.—CBA mouse 96, treated for 10 months. Leiomyosarcoma, showing characteristic bundles cut in various directions. × 100.

FIG. 6.—Another field from the same tumour as in Fig. 5, showing hyperplastic, proliferating endometrial glands, surrounded by sarcoma. × 100.

FIG. 7.—White mouse 7, treated for 5 months. Hyperplastic endometrial glands surrounded by sarcoma. There are collections of polymorphonuclear leucocytes in two glands. × 100.

FIG. 8.—CBA mouse 104, treated for 9 months. Endometrial adenocarcinoma composed of irregularly disposed acini and abundant fibrous stroma. × 100.

FIG. 9.—CBA mouse 98, treated for 16 months. Endometrial adenocarcinoma composed of large and small glandular acini. × 100.

FIG. 10.—Another field from the same tumour as in Fig. 9, showing squamous metaplasia of the glandular elements. × 100.

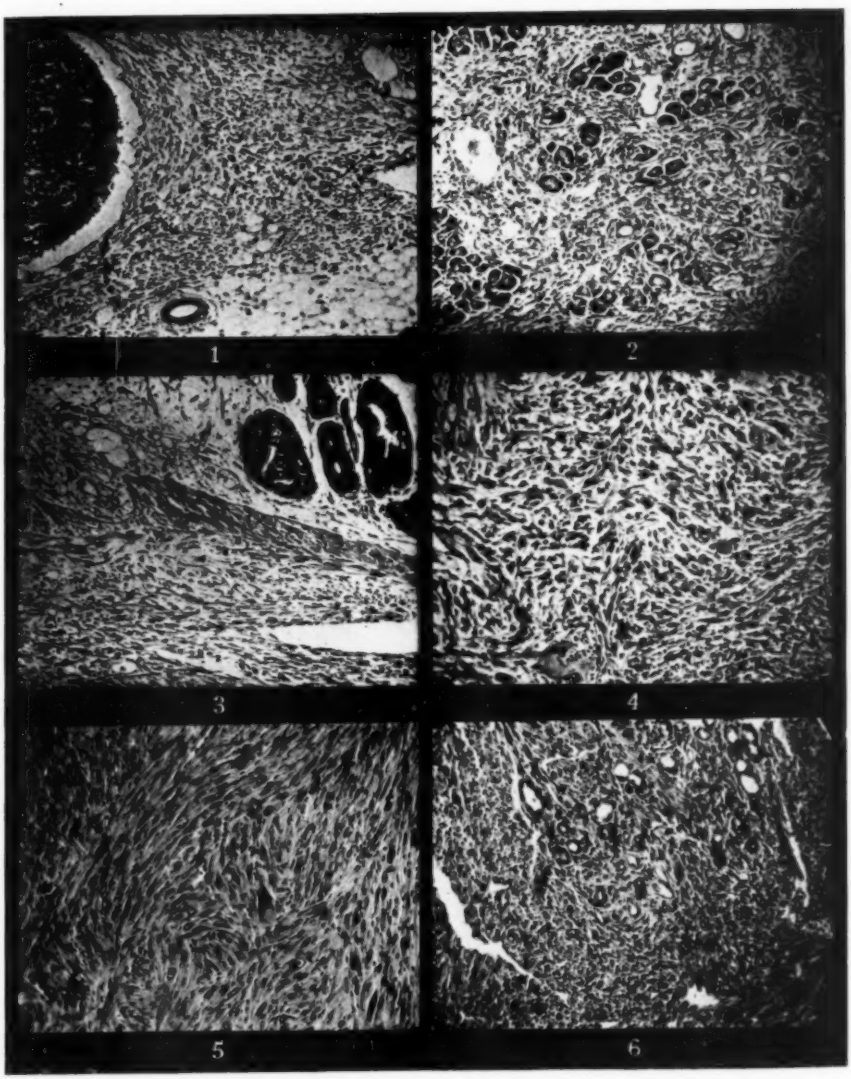
FIG. 11.—White mouse 23, treated for 12 months. Above, the tumour is a characteristic papillary adenocarcinoma; below there is advanced squamous metaplasia. × 42.

FIG. 12.—CBA mouse 99, treated for 6 months. The tumour is a pure squamous cancer. × 39.

FIG. 13.—Metastasis in regional lymph gland of glandular carcinoma shown in Fig. 11. × 90.

FIG. 14.—Immature CBA mouse, 11 g. in weight, untreated. Transverse section of uterine horn, showing absence of endometrial glands. × 40.

FIG. 15.—White mouse 118, treated for 15½ months. The left horn (i.e. the implanted horn) of a spayed immature mouse showing absence of endometrial glands. × 40.



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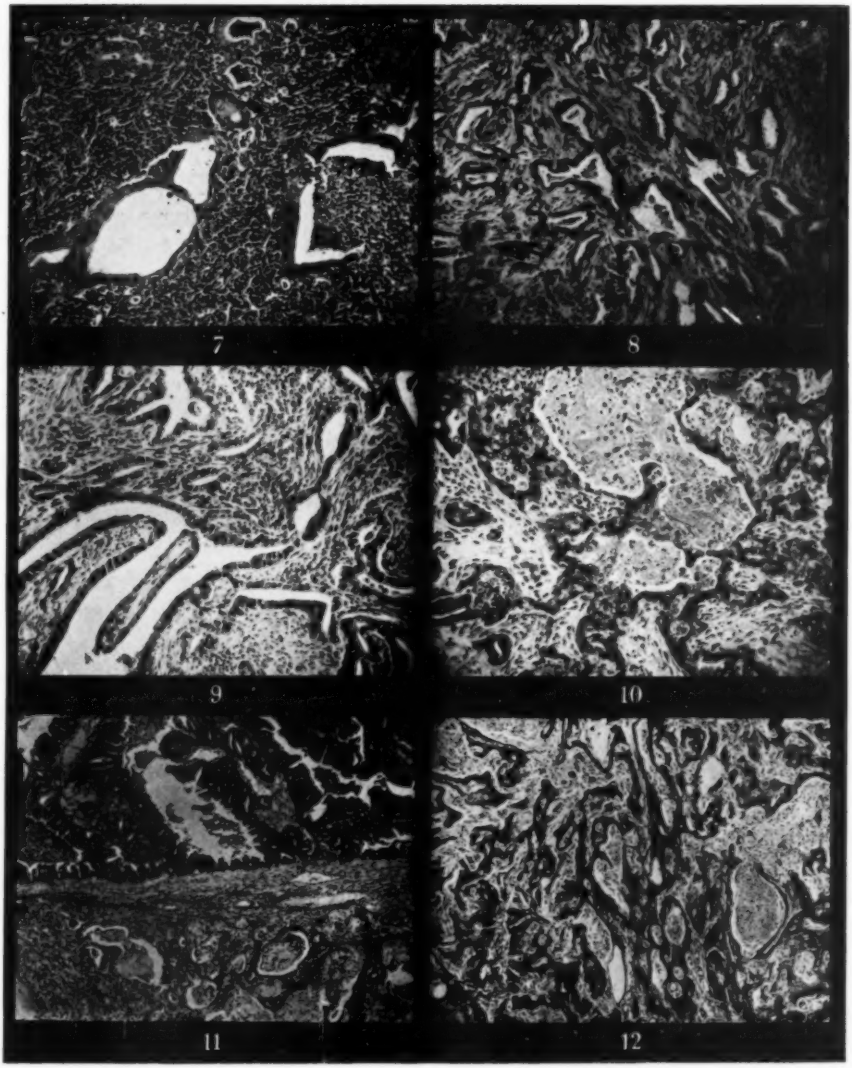

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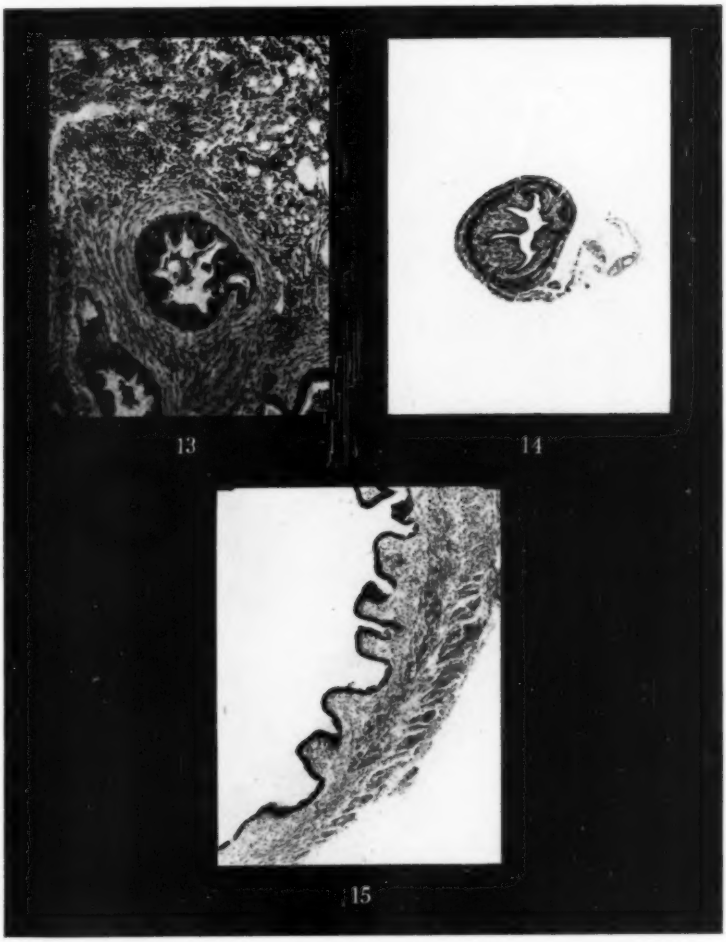

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TABLE III.—*Comparison of Tumour Incidence in CBA and White Mice.*

	Number of mice.	Carcinoma. Number.	Carcinoma. Per cent.	Carcinoma. Average induction period (months).	Sarcoma. Number.	Sarcoma. Per cent.	Sarcoma. Average induction period (months).
Immature CBA	15	6	40·0	10·5	8	53·3	8·0
All types white	94	14	14·9	11·5	7	7·4	8·0
Immature white	36	3	8·3	10·2	3	8·3	5·8

compared with 11·5 months in white mice), but the induction period of sarcomas was similar in the two groups (8 months). The range of induction period of carcinomas was also greater in white mice (4½–20 months ; Table II).

Effect of degree of maturity at the time of implantation.

It was rather surprising that carcinoma could be induced by this means in immature mice, spayed at the time of implantation, when they weighed 11–15 g. (white mouse 85, CBA mice 103, 104 and 107 ; Table I), where the uterine glands could be expected to be in a state of minimum activity (Fig. 14, 15). It is evident, however, that maturity at the start tended to improve the tumour yield, though it did not lower the period of induction in white mice (Table IV). This factor could not be tested on CBA mice, as only immature mice were used.

Effect of spaying.

Spaying had no apparent effect on the incidence of all tumours either in CBA mice (Table I), where the numbers were very small, or in white mice (Table IV).

TABLE IV.—*Influence of Spaying, Maturity and Technique on the Incidence of Tumours in White Mice.*

Type of mouse.	Number.	Carcinoma.	Sarcoma.	Carcinoma and sarcoma.	Percentage of mice with tumours.	Average period after implantation of appearance of tumours.
Spayed	45	6	1	—	16 ± 8·5	10 months.
Intact	49	7	5	1	27 ± 8·5	11 „
Immature	36	2	2	1	14 ± 8·1	8·6 „
Mature	58	11	4	—	26 ± 8·1	11·3 „
Crystal implantation	63	9	6	1	25 ± 8·1	11·4 „
Lard injection	31	4	—	—	13 ± 8·1	8 „

The yield of sarcomas was, however, greater in intact than in spayed white mice, though the difference was not statistically significant. But when white mice implanted with crystals, i.e. mice which received the larger dose of carcinogen, are considered (Table V), it is noteworthy that the lowest incidence of tumours occurred in spayed immature mice. Even though the numbers are small, the agreement between the three other groups in Table V suggests that the lower incidence of tumours in spayed immature mice is of significance.

Effect of technique.

The implantation of crystals of methylcholanthrene was rather more effective in inducing tumours than was the introduction of a 1 per cent solution in lard

TABLE V.—*Influence of Spaying and Maturity on Tumours in White Mice Implanted with Crystals.*

Type of mouse.	Number.	Carcinoma.	Sarcoma.	Carcinoma and sarcoma.	Percentage of mice with tumours.	Average period after implantation of appearance of tumours.
Spayed mature	12	3	1	—	33 ± 11·6	11 months.
Intact mature	24	5	2	—	31 ± 11·6	13·5 „
Spayed immature	14	1	—	—	7 ± 11·6	—
Intact immature	13	1	2	1	31 ± 11·6	6·4 „

(Table IV), though again the difference was not statistically significant; the induction period was not reduced. The weight of crystals (0·1 mg.) was very much less than that used by Castellano and D'Amour (1944) and Greenblatt and Kupperman (1947) in the rat (10 mg.). It is probably comparable with the dose used by Pan and Gardner (1948) for the induction of tumours in subcutaneous implants (" small crystals ").

Histological type.

The carcinomas were classified as adenocarcinomas, adenocarcinomas with squamous metaplasia and as frank squamous carcinomas. Neither strain, degree of maturity at the time of implantation, spaying nor technique affected the type of cancer induced. Polymorphonuclear exudate was a very common accompaniment of all types of carcinoma, sometimes proceeding to pyometra. Pyometra was frequently observed in those uterine horns is which no cancer developed.

SUMMARY.

The experiment has shown that direct implantation of a carcinogen into the uterine horn of the mouse is a suitable method for inducing endometrial carcinoma. A high incidence may be expected in mice of the CBA strain, and quite possibly also in mice of other strains not yet tested. The picture is somewhat complicated by the occurrence of sarcomas, which tend to occur a little earlier than carcinomas, but improvement in technique might obviate this difficulty. There is evidence that hormonal factors are to some extent concerned in the induction of endometrial carcinoma by this means.

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SQUAMOUS CARCINOMA OF THE FORESTOMACH AND OTHER LESIONS IN MICE FOLLOWING ORAL ADMINISTRATION OF 3:4:5:6-DIBENZCARBAZOLE.

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3:4:5:6-Dibenzcarbazole has been tested on numerous occasions and has been shown to be a potent carcinogen in mice (Boyland and Brues, 1937; Strong, Smith and Gardner, 1937; Boyland and Mawson, 1938; Andervont, 1939; Andervont and Edwards, 1941). The results are summarized in Table I. Benign and malignant cutaneous tumours were observed after skin painting and subcutaneous sarcomas after injection. In both types of experiment distant tumours were found in the lungs and liver. In the latter organ the changes were very severe and comprised cirrhosis and bile-duct proliferation, which proceeded to the formation of cholangiomas, as well as hepatomas.

As Boyland and Brues (1937) had suggested that naphthylamine workers might come in contact with dibenzcarbazoles during the course of their work, it was decided to re-investigate 3:4:5:6-dibenzcarbazole as a potential bladder carcinogen. For this purpose it seemed probable that much larger doses than those used hitherto would be required. Mice of the CBA strain were chosen, as this strain had previously given a higher yield of bladder tumours when 2-acetylaminofluorene was fed by stomach tube than four other strains tested (Armstrong and Bonser, 1947). Later, mice of the Strong A strain were also included, as the livers of these mice suffered the least damage in the above experiment.

METHOD.

Mice of the two strains CBA and Strong A were used, having been brother-sister bred in the laboratory. At the age of 12 weeks administration of 3:4:5:6-dibenzcarbazole, supplied by Professor E. Boyland (Chester Beatty Research Institute, Royal Cancer Hospital, London), in arachis oil by stomach tube twice per week was commenced. The technique of this method of administration is described by Armstrong and Bonser (1944). As the mortality in CBA mice in the first few weeks was very high, successive groups were tested at different dosage until a reasonable survival was obtained (Table II). The CBA mice were treated until death; in Strong A mice, when it was evident from the swollen state of the abdomen that much liver damage had taken place, treatment was continued for as long as the mice seemed able to support it and was then withheld until death, the interval in some cases being over 20 weeks (Table III). By this means it was possible to prolong the life of both sexes into the period 50 to 60 weeks.

The bladder was inspected with the naked eye or with the dissecting microscope

TABLE I.—*Summary of Results of Previous Workers.*

Author.	Mice tested. Number.	Type.	Sex.	Neoplasms observed.	Approximate time range.	Mode of administration.	Dose. (mg.)
Boyland and Brues (1937)	26	Stock mice, Simpson strain	..	4 papilloma skin, 4 carcinoma skin	100–321 days	Skin painting (0·3% reduced to 0·1%)	4·2 (approx.) 4·8
	25	„	..	4 carcinoma skin, 5 hepatomas	150–321 „	Skin painting (0·1%)	2·4 6·2
Strong, Smith and Gardner (1937)	5	CBA mice	♂	4 carcinoma skin,* 1 spindle cell sarcoma	Several months	Skin painting (0·5%)	Not known
	18	„	♂	14 subcutaneous sarcomas, 5 hepatomas, 2 pulmonary tumours	204 days	6 subcutaneous injections	1·2
	24	„	♂	24 subcutaneous sarcomas, 3 hepatomas	145 days	„	1·2
	18	A mice	♂	17 subcutaneous sarcomas	138 days	„	1·2
Boyland and Mawson (1938)	28	Stock mice	..	1 sarcoma at site of injection	176 days	1 intraperitoneal injection	0·25
	83	„	..	33 bile duct proliferation or cholangioma	35–200 days	„	0·25
Andervont (1939)	20	A mice	♀	78% pulmonary tumours	4·2 months	1 subcutaneous injection	0·2
Andervont and Edwards (1941)	170	„	♀	45·4% subcutaneous sarcomas, 67·8% pulmonary tumours	10–42 weeks „	1 subcutaneous injection	0·2
	83	„	♂	55·3% subcutaneous sarcomas	„	„	0·2
	11	C3H mice	♀	45·5% „	„	„	0·2
	49	„	♂♀	41·5% „	„	„	0·2
	23	C mice	♂♀	47·8% „	„	„	0·2
	44	„	♂	79·5% „	„	„	0·2

* Including one carcino-sarcoma.

in all cases, previous experience having shown that it is not difficult to detect early bladder lesions by these means. A microscopical examination was also made in approximately one-quarter of the mice.

RESULTS.

Mortality.—In the early weeks the death-rate of CBA females in Experiments I to III was much greater than that of males (Table II). Even when, in Experiment III, the dose was reduced to 0·5 mg. per week, a level fairly well tolerated by the males (Experiment II*b*), all the females died before the expiration of 5 weeks. The cause of death was peripheral lobular liver cell necrosis, upon which pneumonia supervened. The dose was not found which would permit the females to live for more than 20 weeks, although 27 out of 60 CBA males survived from 20 to 49 weeks.

TABLE II.—*Five Experiments in which* 3:4:5:6-*dibenzcarbazole was Administered to CBA and Strong A Mice in Solution in Arachis Oil by Stomach Tube Twice Weekly.*

Expt.	Number of mice. F.	Number of mice. M.	Treatment.	Dose per week mg.	Deaths. F.	Deaths. M.	Survival (weeks.)
Ia	13	15	0·2 c.c. of 1 per cent solution reduced after one week to 0·1 c.c. of 1 per cent solution	4	11	1	1
				2	2	14	3–12
Ib	..	9	0·1 c.c. of 1 per cent solution reduced after 7 weeks to 0·1 c.c. of 0·5 per cent solution	2	0	3	0–9
				1	0	3	10–19
					0	3	20–39
IIa	9	11	0·1 c.c. of 0·5 per cent solution	1	8	4	0–9
					0	1	10–19
					1	6	20–29
IIb	..	10	0·1 c.c. of 0·5 per cent solution reduced at varying times to 0·1 c.c. of 0·25 per cent solution	1	0	2	10–19
					0	4	20–29
				½	0	4	30–49
III	14	..	0·1 c.c. of 0·25 per cent solution	½	14	..	0–5
IV	13	15	0·1 c.c. of 0·125 per cent solution increased after 6 weeks to 0·1 c.c. of 0·25 per cent solution	¼	9	2	0–9
					4	3	10–19
				½	0	2	20–29
					0	6	30–39
					0	2	40–49
V	18	12	0·1 c.c. of 0·125 per cent solution increased after 6 weeks to 0·1 c.c. of 0·25 per cent solution	¼	5	1	0–9
					1	1	30–39
				½	1	6	40–49
					11	4	50–59

In experiments I to IV, CBA mice were used. In experiment V, Strong A mice were used.

In Experiment V, Strong A female mice showed only a slight tendency to die early from the effects of the chemical with the dose given, 5 out of 18 dying in the first few weeks, but when once tolerance had been established the females survived rather longer than the males (Table II).

Bladder.—No epithelial changes were observed.

Forestomach.—Squamous papillomata of the forestomach were observed in a CBA male mouse after 17 weeks of treatment. In Table III are listed all the

TABLE III.—*Occurrence of Gastric and Liver Tumours.*

Strong A Mice.										CBA Mice.									
Mouse number.	Sex.	Weeks of survival.	Total dose of chemical (mg.).	Stomach.			Liver.			Mouse number.	Sex.	Weeks of treatment.	Total dose of chemical (mg.).	Stomach.			Liver.		
				No tumour.	Pap.	Carc.	No tumour.	Benign hep.	Mal. hep.					No tumour.	Pap.	Carc.	No tumour.	Benign hep.	Mal hep.
1	♀	36 (35)	16·0	..	+	..	..	+	..	1	♂	17	7·0	..	+	..	..	+	..
2	♀	45 (32)	14·5	..	+	..	..	..	+	2	♂	19	8·0	0	..	..	0	..	..
3	♀	51 (35)	16·0	..	..	+	..	..	+	3	♀	20	8·5	..	+	..	0	..	..
4	♀	51 (35)	16·0	..	+	..	..	..	+	4	♂	20	8·5	0	..	..	0	..	..
5	♀	51 (35)	16·0	..	+	..	..	..	+	5	♂	20	8·5	0	..	..	0	..	..
6	♀	52 (31)	14·0	..	+	..	..	..	+	6	♂	21	9·0	0	..	..	0	..	..
7	♀	52 (31)	14·0	0	..	..	..	..	+	7	♂	22	9·5	0	..	..	0	..	..
8	♀	53 (32)	14·5	..	+	..	0	..	..	8	♂	23	10·0	..	..	+	..	..	+
9	♀	55 (44)	20·5	..	+	..	0	..	..	9	♂	24	10·5	..	+	..	0	..	..
10	♀	56 (44)	20·5	..	+	..	..	..	+	10*	♂	25	11·0	..	+	..	0	..	..
11	♀	56 (44)	20·5	..	+	..	..	..	+	11	♂	25	11·0	0	..	..	0	..	..
12	♀	56 (44)	20·5	..	+	..	..	+	..	12	♂	25	11·0	..	+	..	..	+	..
13	♀	56 (44)	20·5	..	..	+	..	..	+	13	♂	27	12·0	..	+	..	..	+	..
14	♂	36 (36)	16·5	..	..	+	..	+	..	14	♂	27	12·0	..	..	+	..	+	..
15	♂	45 (45)	21·0	..	+	..	..	..	+	15	♂	27	12·0	..	+	..	..	+	..
16	♂	45 (45)	21·0	0	..	..	..	..	+	16	♂	29	13·0	..	+	..	..	+	..
17	♂	47 (43)	20.0	..	+	..	..	..	+										
18	♂	48 (44)	20·5	..	+	..	..	+	..	17	♂	29	13·0	0	..	..	0	..	..
19	♂	49 (49)	23·0	..	+	..	..	..	+	18	♂	30	13·5	..	+	..	..	+	..
20	♂	49 (49)	23·0	..	+	..	..	..	+	19	♂	30	13·5	..	+	..	0	..	..
21	♂	51 (37)	17·0	0	..	..	..	..	+	20	♂	30	13·5	..	+	..	..	+	..
22	♂	52 (37)	17·0	0	..	..	..	..	+†	21	♂	34	15·5	..	+	..	..	+	..
23	♂	52 (48)	22·5	..	+	..	..	..	+	22	♂	34	15·5	0	..	..	..	+	..
24	♂	59 (37)	17·0	..	+	..	..	..	+	23	♂	37	17·0	..	+	..	..	+	..
										24	♂	37	17·0	0	..	..	0	..	..
										25	♂	38	17·5	..	..	+	..	+	..
										26	♂	38	17·5	..	+	..	..	+	..
										27	♂	39	18·0	..	+	..	0	..	..
										28	♂	39	18·0	..	+	..	0	..	..
										29	♂	41	19·0	..	+	..	..	+	..
										30	♂	46‡	19·0	..	..	+	..	+	..
										31	♂	49	23·0	..	+	..	0	..	..
24	13 ♀ 11 ♂	Av. = 50·1 (40·0)	Av. = 17·8	4	17	3	2	4	18	31	1 ♀ 30 ♂	Av. = 29·9	Av. = 13·3	9	18	4	15	15	1

Figures in brackets denote weeks of treatment.
Pap. = papilloma.
Carc. = carcinoma.
Hep. = hepatoma.
* This mouse had a pleomorphic sarcoma of the muscles of the posterior abdominal wall.
† There were secondary deposits in the lung from this tumour.
‡ This mouse survived for 5 weeks after the last treatment was administered at 41 weeks.

mice of both strains which were treated or survived for periods of from 17 to 59 weeks. Of 55 mice, 23·6 per cent showed no tumours, 63·6 per cent had papillomatosis and 12·7 per cent frank squamous carcinomas. The number of papillomata ranged from 1 to 14 or 15 and the size from pinhead to as much as 7 or 8 mm. in diameter. All were fronded, and on microscopical examination were well-differentiated keratinizing squamous papillomas, with a delicate connective-tissue core (Fig. 1). Several showed such a degree of cellular irregularity in the basal layers as to be consistent with a diagnosis of early malignancy, but this diagnosis was not made unless there was invasion and destruction of the muscular coat of the stomach of such degree as to leave no doubt as to the invasiveness of the process. All the 7 carcinomata were of squamous type (Fig. 2), the largest in Strong A male 14 attaining a size of 2·0 × 1·4 × 1·4 cm. In CBA Mouse 25 there was extensive spread of cancer *via* the serosal lymphatics (Fig. 3). These proliferative changes were entirely confined to the forestomach, the glandular stomach showing on occasion inflammatory reactions, but no hyperplasia. Nor was any proliferative lesion of the rest of the intestinal tract observed.

Liver.—The livers of all the mice suffered severely. Many of the females dying during the first few weeks of treatment showed deep yellow staining of all tissues and organs and this was attributed to jaundice. Even at 3½ weeks early cellular cirrhosis and bile-duct proliferation was observed. By 20 weeks all the changes were well established, including nodular regeneration of the parenchymal cells ; in mice dying after 40 weeks of treatment they were very advanced (Fig. 4). The livers of all the mice of both strains treated for 20 weeks or more showed bile-duct cystadenomas ; in Strong A Mouse 12 and CBA Mouse 28 the condition had progressed to malignancy (Fig. 7). Finally hepatomas occurred (Table III), some of which were undoubtedly malignant, metastases to the lungs being observed in Strong A Mouse 22 (Fig. 5, 6). The livers were on the whole larger in Strong A than in CBA mice, but it was thought that some reduction in liver size occurred in the mice of the former strain when the treatment was withdrawn.

Lungs.—Apart from terminal pneumonia, no hyperplastic nor other changes were noted in the lungs of CBA mice. However, in every Strong A mouse listed in Table III pulmonary adenomas were observed. No attempt was made to count or measure these tumours, but the impression was gained that they were, on the whole, of larger size in those mice which were treated until death than in those in which a long interval elapsed between the cessation of treatment and death.

Kidneys.—In CBA mice there was evidence of chronic glomerulo-nephritis from 7 weeks onwards. Increased cellularity of the tufts was followed by hyalinization ; metaplasia of Bowman's capsule to a cuboidal type of epithelium also occurred. There was focal lymphocytic infiltration of the interstitial tissue, proceeding to fibrosis, and after 20 weeks of treatment there was usually calcification of some of the cortical tubules and of the adjacent glomerular vessels. The kidneys of 4 Strong A mice were examined microscopically and all were normal.

Posterior abdominal wall.—In CBA Mouse 10 a smooth white fleshy tumour was found at *post-mortem* arising from the muscles of the posterior abdominal wall behind the rectum. It was invading through the anterior abdominal wall into the thigh muscles. It was not connected with rectum, testes, bladder,

† There were secondary deposits in the lung from this tumour.
‡ This mouse survived for 5 weeks after the last treatment was administered at 41 weeks.

ureter nor kidneys. On microscopical examination the tumour had the structure of a spindle-cell sarcoma, in which were very numerous multinucleated giant cells of bizarre type.

DISCUSSION.

No changes were observed in the bladders of mice of two strains after prolonged and heavy dosage with 3:4:5:6-dibenzcarbazole. It is to be noted, however, that no CBA mouse survived longer than 49 weeks, while all but one Strong A mouse survived into the period 40 to 59 weeks. At 49 weeks only one of 18 CBA mice treated with the potent bladder carcinogen 2-acetylaminofluorene was known to have developed a tumour (Armstrong and Bonser, 1947), whereas 5 out of 10 Strong A mice were known to have hyperplasia or a benign papilloma when dying between 40 and 59 weeks. Thus, while it cannot be stated with certainty that 3:4:5:6-dibenzcarbazole administered orally is not a bladder carcinogen, no indication has been obtained which would suggest that it has any action on the bladder epithelium, although from previous experience with these strains of mice evidence of hyperplasia or papillomatosis might be expected to be forthcoming within the experimental period available.

An interesting unexpected finding was the high yield of tumours of the forestomach in both strains (Table III). More Strong A than CBA mice had tumours at death, but the difference is not statistically significant, and may be due to the greater dose of chemical and the longer survival period in Strong A than in CBA mice (Table IV). When it is taken into account that the incidence of carcinomas was identical in the two strains, despite the shorter survival period and smaller dose in CBA mice, it would appear probable that CBA is the more susceptible strain. From Table III it is seen that these tumours of the forestomach are not dependent upon the continued administration of the carcinogen for the maintenance of their growth, as the chemical was discontinued for an average of 10·1 weeks before the death of Strong A mice, the range being 0 to 22 weeks.

It is rather surprising that papillomas of the forestomach were so rarely observed when tar was applied to the skin of mice, Twort and Twort (1932) having observed only occasional papillomas in 60,000 mice so treated. Much

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EXPLANATION OF PLATES.

FIG. 1.—CBA Mouse 8, treated for 23 weeks. Longitudinal section through stomach showing fore and glandular portions. On the left, three large squamous papillomata and another tiny one on the limiting ridge ; on the right, the glandular stomach, free of tumours. One of the papillomata (not shown here) had invaded through the muscular wall of the stomach and was regarded as a squamous cancer. × 3.

FIG. 2.—CBA Mouse 25, treated for 38 weeks. Keratinizing squamous cancer, which has invaded through the muscular wall of the forestomach (lower margin). × 130.

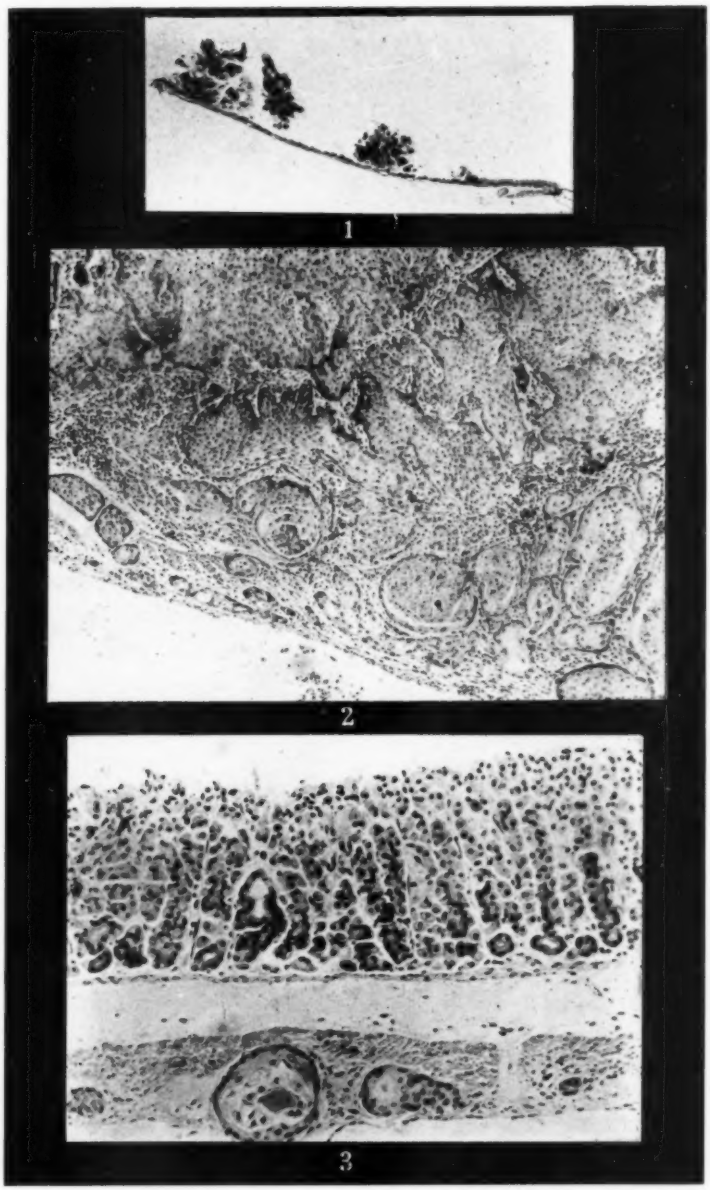
FIG. 3.—Same mouse as in Fig. 2. Invasion of subserosal lymphatics of the glandular stomach by squamous cancer of the forestomach. × 130.

FIG. 4.—CBA Mouse 30, treated for 41 weeks and killed 5 weeks later. Biliary cystadenoma with cirrhosis. There is a group of hyperplastic bile ducts above, and a regenerative nodule of liver parenchyma below. × 55.

FIG. 5.—Strong A Mouse 22, treated for 37 weeks and killed 15 weeks later. Malignant hepatoma showing finger-like columns of tumour cells, which are highly vacuolated, and irregularity of nuclear size. × 130.

FIG. 6.—Same mouse as in Fig. 5. Atrium of bronchus with two deposits of malignant hepatoma cells in peribronchial lymphatic vessels. × 130.

FIG. 7.—Strong A Mouse 12, treated for 44 weeks and killed 12 weeks later. Acini of malignant cholangioma with cellular cirrhosis between. × 130.



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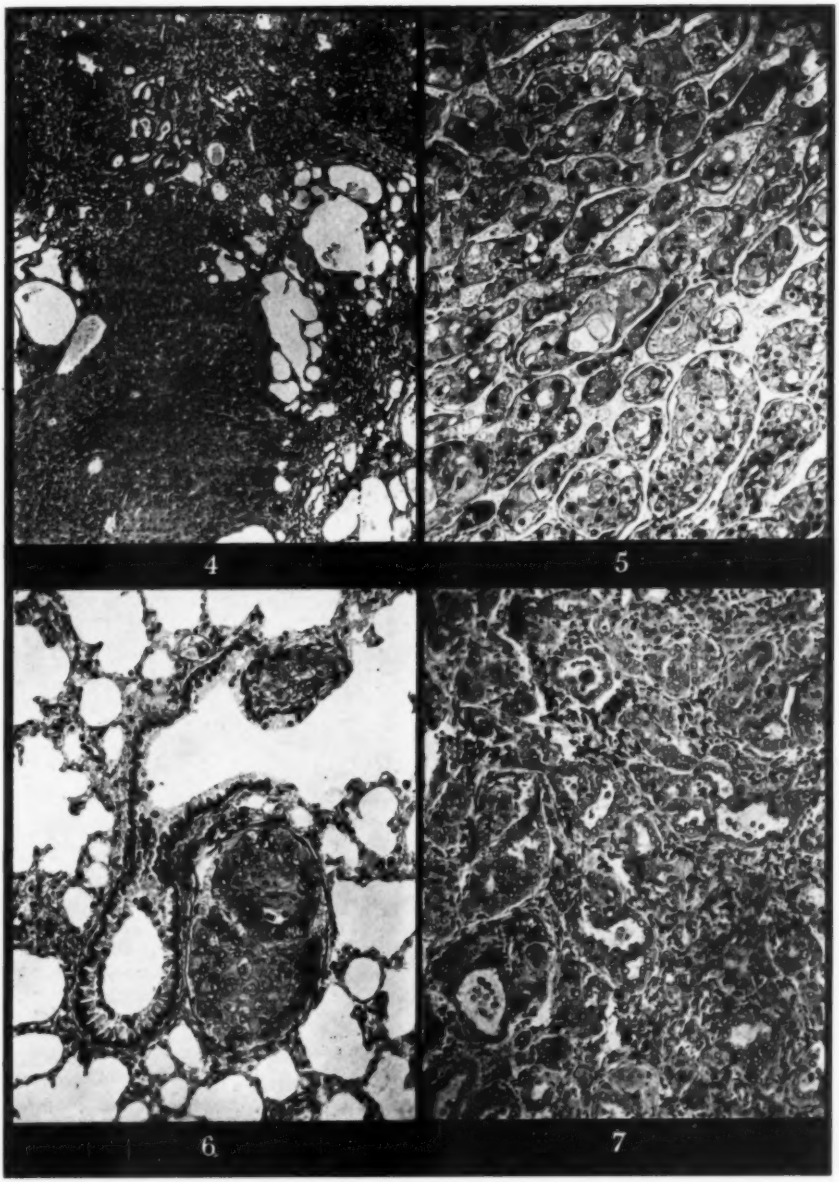

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has been written about the induction of tumours of the forestomach by means of ingestion of carcinogenic hydrocarbons, reviews by Klein and Palmer (1941), Sugiura (1942), Kirby (1943) and Beck (1946) giving a complete account of the subject. It seems clear that 20-methylcholanthrene, 3:4-benzpyrene and 1:2:5:6-dibenzanthracene are carcinogenic in this sense, the latter having produced one benign tumour (Beck, 1946) and several malignant ones (Lorenz and Stewart, 1948). Exhibition in vegetable or mineral oil or in aqueous emulsions or in aqueous soap solution is effective. The addition of 3:4:5:6-dibenzcarbazole may now be made to this list. From the observations so far reported it is necessary to administer the chemical by the oral route, as no similar tumours were found after skin painting or subcutaneous injection. It is concluded that the chemical acts locally on the epithelium of the forestomach.

TABLE IV.—*Incidence of Forestomach Tumours, with Average Survival and Dosage of the Mice.*

		Strong A.	CBA.
		%	%
Tumour incidence	No tumour	16·7	29·0
	Papilloma	70·8	58·0
	Carcinoma	12·5	13·0
		Weeks.	Weeks.
Average survival	No tumour	50·0	25·2
	Papilloma	50·6	31·2
	Carcinoma	47·7	33·6
		mg.	mg
Average dose per mouse	No tumour	17·3	11·1
	Papilloma	18·9	14·2
	Carcinoma	17·7	14·6
Minimum dose per mouse	Papilloma	14·5	7·0
	Carcinoma	16·0	10·0
Maximum dose per mouse	No tumour	21·0	17·5

No tumours of the glandular stomach were observed. The reasons given by Beck (1946) may account for this : the presence of the mucus barrier, the mucus flow tending to remove the carcinogen from contact with the cells, the possibility that contact between a carcinogen and a resting cell may not be sufficient to induce neoplasia, and finally the possibility that glandular epithelium is essentially less susceptible than squamous epithelium to the type of carcinogen under discussion. There seems to be little support for the latter suggestion when the recent work (Horning, 1946 ; Pan and Gardner, 1948) on the induction of tumours by carcinogens in glandular epithelia in implants is considered. Carcinomas of the intestine were not observed, in contrast with the results of Lorenz and Stewart (1946), who noted such tumours in Strain A and other mice which received aqueous emulsions of 20-methylcholanthrene and 1:2:5:6-dibenzanthracene.

Amines are not without a similar action on the epithelium of the forestomach, papillomas having been observed in one RIII, one CBA and 2 Strong A mice at 77, 74, 69 and 65 weeks respectively of treatment with 2-acetylaminofluorene (Armstrong and Bonser, 1947). In addition, Otsuka (1935) described papillomatosis of the forestomach in mice following oral administration of diaminoazobenzene.

One spindle-cell sarcoma of the posterior abdominal wall was observed. It

seems possible that perforation of the stomach might have occurred during intubation, resulting in a false passage of the tube into the muscles of the posterior abdominal wall and consequent injection of the chemical at this site. In the course of observation of the CBA strain for 17 years (unpublished observation), one spontaneous spindle-cell sarcoma has been found and, therefore, the possibility of a spontaneous origin of the tumour in Mouse 10 cannot be completely excluded.

The pulmonary tumours observed in strain A mice were similar to those described by Andervont (1939).

Thus neoplasia may be expected whenever 3:4:5:6-dibenzcarbazole comes in contact with the tissues in sufficient concentration. Its general use as a carcinogen has no doubt been prevented by its severe toxic action on the liver, especially in females. Contrary to the experience of Andervont and Edwards (1941), this sex difference was observed only in the early weeks of the present experiment in strain A mice (Table III).

In comparing the degree of liver damage in the two strains, it is evident that both suffered severely, but that the incidence of hepatomas was greater in strain A mice than in CBA, the proportion of histologically malignant tumours being also greater. It is probable that this was due to the longer survival and greater dosage of the chemical in the former strain (Table IV). The contrast between the liver lesions induced by 2-acetylaminofluorene and 3:4:5:6-dibenzcarbazole is very striking. Not only were hepatomas more abundant and more malignant in Strong A mice than in CBA with the latter chemical, but cirrhosis and bile-duct proliferation were a marked feature in both strains. Although a high incidence of hepatomas was observed in CBA mice (but not in Strong A mice) when treated with 2-acetylaminofluorene (Armstrong and Bonser, 1947), cirrhosis was absent and bile duct changes were minimal. It would appear possible that the chief action of 2-acetylaminofluorene is to enhance the tendency to hepatoma already hereditarily present in CBA mice, whereas 3:4:5:6-dibenzcarbazole damages the liver parenchyma in such a way that changes are set in train which eventually lead to tumour formation (Orr, 1940).

SUMMARY.

An experiment is described in which mice of the Strong A and CBA strains received by stomach tube, twice weekly, an oily solution of 3:4:5:6-dibenzcarbazole, with a view to testing this substance as a bladder carcinogen. The mortality was very high until the dose was reduced to 0·5 mg. per mouse per week, the cause of death being necrosis of the liver parenchyma. Females were more susceptible than males, CBA females being more susceptible than Strong A.

No bladder epithelial changes were observed during an experimental period which would have sufficed to show, in these strains, hyperplasia and early tumour formation had 2-acetylaminofluorene been the carcinogen.

Papillomatosis and carcinomas of the forestomach were observed in both sexes of both strains. The extreme liver changes observed by previous authors were also seen.

We wish to thank Professor E. Boyland for his generous gift of 3:4:5:6-dibenzcarbazole.

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BERYLLIUM BONE SARCOMATA IN RABBITS.

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THE development of bone tumours in rabbits following the intravenous injection of suspensions of synthetic zinc beryllium silicate and beryllium oxide was reported briefly by Gardner and Heslington in 1946. They gave their rabbits 20 injections totalling 1 g. of particles of 3 μ diameter or less during a 6-weeks period. Seven rabbits survived the injections for 7 months or more, and all developed malignant osteosarcomas, often with multiple primary sites.

We have repeated these experiments, using zinc beryllium silicate and beryllium silicate and confirm the findings of Gardner and Heslington (1946), although the proportion of survivors developing tumours is less in our series. Similar confirmation comes in preliminary reports from Cloudman, Vining, Barkulis and Nickson (1949) and Nash (1950). This paper records in greater detail the pathological characteristics of these tumours.

MATERIALS.

Zinc beryllium silicate is manufactured by mixing the component oxides in molecular proportions, and the resultant mixture is fired for 3 to 4 hours at 1250° C. The cooled mass is ground to a fine powder. X-ray analysis has shown that zinc beryllium silicate is really a solid solution of Zn_2SiO_4 and Be_2SiO_4. An analysis of the zinc beryllium silicate used in Experimental Groups B and C (Table I) gave a composition ZnO 67 per cent, SiO_2 31 per cent, BeO 2 per cent. The complex injected in Experimental Group A (Table I) contained manganese, and had a composition ZnO 67 per cent, SiO_2 28 per cent, BeO 2 per cent, MnO 3 per cent. The method of preparation and analysis of the phosphors was described by McKeag and Ranby (1947).

The zinc beryllium silicate, beryllium silicate and zinc silicate were obtained as finely ground powders with a particle size of 5 μ or less in diameter. Before injection these powders were made up as suspensions in water. These silicates were free from metallic contaminants because a high degree of purity is needed to obtain the pure colours in fluorescent lamps in which they are used. The samples of the silicates used in these experiments were examined for radioactivity

TABLE I.—*Summary of Silicates Injected and Fate of Injected Rabbits.*

	Experimental group.					
	A.	B.	C.	D.	E.	F.
Material injected	ZnBe silicate	ZnBe silicate	ZnBe silicate	Beryllium silicate	Beryllium silicate	Zinc silicate.
Concentration of suspension, %	10	30	10	20	10	20
Number of injections	10	6	10	6	10	6
Total amount injected (in g.)	1·0	2·1	1·0	1·2	1·0	1·2
Initial number of rabbits in group	10	12	12	11	12	10
Survivors of course of injections	3	3	11	3	8	8
Fate of survivors	Rabbit 1: Died, 16 weeks	Rabbit 4: Tumour, 45 weeks	Rabbit 7: Died, 20 weeks	Rabbit 18: Tumour, 39 weeks	Rabbit 21: Missing,	Rabbit 29: Killed, 14 weeks
	Rabbit 2: Died, 17 weeks	Rabbit 5: Tumour, 49 weeks	Rabbit 8: Died, 23 weeks	Rabbit 19: Killed, 65 weeks	Rabbit 22: Died, 22 weeks	Rabbit 30: Killed, 14 weeks
	Rabbit 3: Died, 30 weeks	Rabbit 6: Killed, 65 weeks	Rabbit 9: Tumour, 32 weeks	Rabbit 20: Killed, 65 weeks	Rabbit 23: Died, 24 weeks	Rabbit 31: Killed, 17 weeks
	..	..	Rabbit 10: Tumour, 53 weeks	..	Rabbit 24: Died, 65 weeks	Rabbit 32: Killed, 24 weeks
			Rabbit 11: Tumour, 61 weeks	..	Rabbit 25: Killed, 100 weeks	Rabbit 33: Killed, 61 weeks
			Rabbit 12: Died, 71 weeks	..	Rabbit 26: Died, 114 weeks	Rabbit 34: Killed, 72 weeks
			Rabbit 13: Tumour, 83 weeks	..	Rabbit 27: Alive, 120 weeks	Rabbit 35: Killed, 100 weeks
			Rabbit 14: Killed, 104 weeks	..	Rabbit 28: Alive, 120 weeks	Rabbit 36: Alive, 120 weeks
			Rabbit 15: Alive, 120 weeks			
			Rabbit 16: Alive, ditto			
			Rabbit 17: Alive, ditto			

in two separate institutions, and reports from both indicated that the silicates were not radioactive.

The rabbits were of mixed breeds and sexes, and except for one group were kept out of doors in small pens. Coccidial infection was present, but incidental deaths among the experimental animals were largely the result of accidental injuries sustained by the animals while fighting.

METHODS.

Aqueous suspensions of the powders were injected twice weekly in 1 ml. amounts into the ear veins of the rabbits. The beryllium silicates were strong irritants, and thrombosis and sclerosis of the ear veins made the later injections difficult. A large number of animals died within ten minutes of an injection. Death was due to a thrombosis spreading down from the ear veins to the heart and enclosing the injection mass.

The survivors of the series of injections were inspected periodically. All incidental deaths were examined carefully, and specimens of liver, spleen, lungs and kidneys were taken for histological examination. The carcase was then boiled, the skeleton separated, and the individual clean bones examined and in some cases X-rayed. The first tumour was recognized only after the bones had been treated in this way, but fresh material for histological examination was obtained from all subsequent bone tumours.

RESULTS.

Incidence of Tumours.

The details of the 6 groups of animals in this experiment are given in Table I.

Twenty-one rabbits survived the injection of beryllium silicates for 30 weeks or more. Bone sarcomas developed in 7 of these. Five animals are alive and apparently normal 120 weeks from the end of the course of injections. The earliest evidence of malignant change was found at 32 weeks, and the latest tumour in this series developed 83 weeks after the last injection of beryllium silicate.

Eight animals of the control series injected with zinc silicate survived the course of injection. Incidental deaths accounted for 4 of these within the first 6 months, but 4 survived more than a year and 1 is still alive and under observation after 120 weeks. No tumours have been found in any of these control animals injected with zinc silicate.

Reactions of Soft Tissues.

Examination of the tissues of animals dying immediately after injection and between 14 and 83 weeks after the injection of the silicates has shown the presence of silicate particles in the lungs, spleen and liver of all injected animals. A few silicate particles are found occasionally in the kidney and adrenal, but these do not produce any reaction. The particles of zinc silicate seen in tissues differ from the beryllium silicates in being rounded, less angular, less refractile, and tending to form aggregates. The beryllium silicate particles are at first scattered, but become aggregated within macrophages and giant cells.

Lungs.—Aggregates of silicate particles occlude the lumen of terminal arterioles and of alveolar capillaries. The particles are associated with an obliterative endarteritis and a proliferation of adventitial cells. The particles become surrounded by macrophages and foreign body giant cells. The small nodules that

result from the macrophage proliferation are scattered throughout the lung. The nodules are fairly uniform in size, about 25 μ in diameter, and often have a hyaline centre. They are not associated with any reticular reaction or collagen formation. The nodules appear to be chiefly in the alveolar septa. They develop within 10 to 20 weeks of injection and persist unchanged for at least 114 weeks.

In the animals injected with the beryllium silicates general increase in fibrous tissue occurs in the lungs. The alveolar septa are thickened by newly formed collagen fibres. These do not usually appear until at least 3 months after injection and thereafter are constantly present. The fibrosis does not appear to be directly associated with the silicate-containing nodules, which remain discrete and free from collaginous coating. The animals injected with zinc silicate may show a slight increase in pulmonary fibrous tissue, but this is less than in animals injected with the beryllium silicates.

Spleen.—Immediately after the injection of the beryllium silicates the particles are widely scattered throughout the red pulp. In the following months most of the particles are picked up and aggregated by the macrophages. The macrophages of the spleen first form giant cells of the foreign body type and even of the Langhans type, but later the particles are contained in large syncytial masses, which show up to 100 nuclei in a single section. The formation of macrophages is associated with a disappearance of the white pulp of the spleen and of the lymphocytes of the red pulp. The spleen shrinks, but there is no true increase in either collagen or reticulum fibres. The spleen is atrophic rather than fibrotic.

The spleen of animals injected with zinc silicate differs in that the normal red and white pulp persists. The macrophage response is the same as after the beryllium silicate injections, and the syncytial masses containing particles are found in both red and white pulp.

Liver.—After injection the particles can be seen either free in the sinusoids or more commonly within the Kupffer cells. For some months the particles are present in isolated Kupffer cells, but 3 to 4 months after injection the Kupffer cells have increased in size and number to form cellular masses that fill and often distend the sinusoids. These masses, which resemble emboli in appearance (Fig. 1), vary between 15 μ and 50 μ in diameter, with a mean diameter of 25 μ. Their distribution in the liver is uniform. They probably arise from the Kupffer cells that originally took up the particles. They are not associated with any reticular or fibrous tissue reaction, and develop after the injection of both the beryllium silicates and zinc silicate.

Bone marrow.—The changes in the bone marrow resemble those in the liver and spleen. Numerous nodules are scattered throughout the marrow of all parts of the skeleton, and are visible on naked eye examination of the divided bones (Fig. 13). Histologically the nodules consist of aggregates of macrophages (Fig. 2), whose cytoplasm is packed with granules of refractile silicate particles (Fig. 3). The nodules are present in all injected animals whose bones have been searched for them, and in many cases are not associated with any other abnormality, either histological or radiological. Identical focal changes in the bone marrow were found in the animals injected with zinc silicate.

Features of Bone Sarcomata.

Malignant tumours have been found in 7 animals, and the details of dosage, time of tumour development and bones involved are summarized in Table II.

TABLE II.—*Rabbits Developing Tumours.*

Number.	Material.	Amount injected (g.)	Time for tumour development. (weeks.)	Sites of tumours.
Rabbit 9	ZnBe silicate	1·0	32	Medullary bone formation in both humeri (Fig. 12), both tibiae and both femora (Fig. 7).
Rabbit 18	Be silicate	1·2	39	Tumours : L. humerus, r. tibia and pelvis. Medullary bone formation R. humerus, L. tibia and both femora.
Rabbit 4	ZnBe silicate	2·1	45	Tumour L. humerus. Medullary bone formation R. humerus, both femora (Fig. 9 and 5).
Rabbit 5	„ „	2·1	49	Tumours R. humerus (Fig. 14 and 6), R. tibia and femur (Fig. 15, 10, 17), R. scapula (Fig. 19). Medullary bone formation L. humerus, both femora, both tibiae. Metastases, lymph nodes, lungs (Fig. 16 and 21), liver, peritoneal and pleural surfaces.
Rabbit 10	„ „	1·0	53	Tumours L. femur (Fig. 8 and 18), R. humerus, L. tibia (Fig. 11). Medullary bone formation in all long bones. Metastases, lymph nodes, lungs and liver.
Rabbit 11	„ „	1·0	61	Tumour and medullary bone formation R. tibia. Metastasis in liver (Fig. 22).
Rabbit 13	„ „	1·0	83	Tumour R. tibia. Medullary bone formation both humeri, L. tibia, L. femur.

The protocols of the individual animals will be considered in detail. Full accounts of the tumours are given, so that their malignant character can be appreciated and their significance assessed.

Rabbit 9.

At autopsy no abnormality in the external contour of the bones was recognized. The diagnosis of malignant bone sarcoma depends entirely on a consideration of radiographs of the macerated skeleton and the diagnosis is retrospective.

In each humerus (Fig. 12) there was abundant bone formation in the upper part of the medullary cavity. In each femur there was similar bone formation in a localized area of the midshaft (Fig. 7). Similar changes were present in the upper part of each tibia. In all these sites the symmetrical distribution of the newly formed bone was remarkable. These findings correspond closely with those for Rabbit 4, where consideration of histological material in conjunction with radiographs leads to the conclusion that the radiographic appearances result from occupation of the marrow cavity by ossifying tumour tissue.

It is therefore assumed that the radiographic appearances shown in Fig. 12 and 7 represent an early stage of tumour development, without expansion of the affected bones or metastasis to other organs.

Rabbit 18.

At autopsy a palpable swelling of the upper part of the left humerus was present, but other bones appeared normal. The tumours were recognized only when the macerated bones were examined and no histological material was available.

Gross tumours were present in the upper part of the right tibia and in each half of the pelvis. As well, there was medullary bone formation in the shaft of the right humerus, the left tibia, and throughout each femoral shaft.

Rabbit 4.

At autopsy this animal presented a bulky tumour of the upper part of the shaft of the left humerus. Most of the tumour tissue has the structure of an anaplastic pleomorphic undifferentiated sarcoma with round and spindle-shaped cells. In some areas the tumour cells resemble those of an epithelial tumour, showing well-marked cytoplasmic outlines, and being arranged in solid masses and branching strands. In a few areas osteoid or bony matrix is present between the spindle-cells of the tumour, this being the only evidence of specific bony differentiation. Tumour invasion of small veins is prominent, and although no gross pulmonary metastases were recognized, sections of lung tissue reveal tumour emboli and early developing metastases. These pulmonary deposits all have an undifferentiated spindle-celled structure.

Medullary bone formation was recognized radiographically in the right humerus and in each femur (Fig. 9). Histological examination of the femoral shaft shows a complete replacement of the normal marrow by tumour tissue, quite comparable to that seen in more advanced lesions in other animals, but not yet expanding the periosteum or causing erosion of the cortical bone of the shaft. Part of the tumour is bony, part cartilaginous, while some areas consist merely of anaplastic spindle-celled tissue. Histological changes in the marrow of the shaft of the humerus where another localized area of medullary bone formation had been recognized radiographically are of great interest. The cortex of the bone is normal, and the greater part of the contained marrow consists of cellular haemopoietic tissue, with the occasional focal aggregates of beryllium-containing macrophages that are present in all these animals. In this bone some of these nodules are quite extensive, forming confluent masses as large as 1·5 mm. in diameter. But in addition to these scattered lesions, an extensive area of marrow fibrosis is present in the shaft of the bone, extending over 1 cm. of its length. Here haemopoietic cells are absent, and the predominant tissue is spindle-celled fibrous tissue. Numerous fat cells are scattered through the area, and the tissue varies from an almost acellular mass of fibres to a tissue composed largely of rounded and elongated fibroblasts. Some of the cells are seen to be in mitosis, and the appearance is that of an actively proliferating tissue (Fig. 4). In some areas the fibrous tissue shows evidence of specific bony differentiation. Fig. 5 shows such an area, where a network of recently-formed bone-trabeculae is present, and where the osteoblastic cells covering the surfaces of the trabeculae are linked to the intervening fibroblasts by numerous intermediate forms. It is these bone-trabeculae which are responsible for the radiographic appearance of medullary bone-formation.

Rabbit 5.

At autopsy a bulky tumour (Fig. 14) of the right humerus was present. Metastases were found in axillary and mediastinal lymph nodes, in lungs (Fig. 16) and liver, and were studded over the peritoneal and pleural surfaces. A smaller tumour of the right tibia was also present, and radiographs of other long bones

demonstrated marked medullary bone formation in the shaft of left humerus, and of each femur and tibia. Scattered focal nodules were present in bone-marrow generally.

Radiologically the tumour of the humerus is a predominantly osteolytic lesion. Histologically it is an anaplastic spindle-celled tumour, with numerous irregular giant-cells scattered throughout (Fig. 6). Osteoid and bony intercellular material is seen only in a few scattered areas. There is no evidence of

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EXPLANATION OF PLATES.

FIG. 1.—A low-power view of the liver, showing several masses of Kupffer cells in both the portal and hepatic tissue. From an animal 45 weeks after injection with zinc beryllium silicate. Although not visible in this picture, these lesions are loaded with refractile silicate particles. × 100.

FIG. 2.—A low-power photomicrograph of an area of fatty bone-marrow from the lesion illustrated in Fig. 13. Numerous rounded "granulomas" are seen. × 40.

FIG. 3.—A high-power view of one of the lesions in Fig. 2, showing masses of refractile crystals. × 270.

FIG. 4.—An area of newly-developed fibrous tissue in the bone-marrow of the shaft of the right humerus in Rabbit 4. × 100.

FIG. 5.—Another area from the marrow cavity of the same bone (Rabbit 4) showing formation of bone trabeculae in the fibrous tissue. × 100.

FIG. 6.—The histological structure of the tumour shown in Fig. 14. Round and spindle-shaped cells predominate, and numerous multinucleated tumour giant-cells are present. × 80.

FIG. 7.—A radiograph of the femur from Rabbit 9, showing a localized area of medullary bone formation just below the mid-point of the shaft. × 2.

FIG. 8.—A radiograph of a slab of tissue from the tumour of the femur in Rabbit 10. The tumour tissue consists almost entirely of fine calcified bone trabeculae. × 2.

FIG. 9.—A radiograph of the femur in Rabbit 4. Subsequent histological examination of this femoral shaft showed a complete replacement of marrow by ossifying tumour tissue. × ¾.

FIG. 10.—A radiograph of a slab of tissue from the bones illustrated in Fig. 15. × 1¼.

FIG. 11.—A radiograph of a slab of tissue from the tumour of the left tibia in Rabbit 10. The part of the lesion expanding the periosteum on the right side of the picture consists of bone-forming tumour tissue. On the left side of the bone a few areas show a similar pattern, but there is also some more diffuse aggregation of radio-opaque material, corresponding to the formation of calcified cartilage by the tumourtissue. These radiographic features were confirmed histologically. × 2.

FIG. 12.—A radiograph of the humerus from rabbit 9, showing medullary bone formation in the upper part of the shaft. × 2.

FIG. 13.—A photograph of the cut surface of a vertebral body in a rabbit 52 weeks after injection with zinc beryllium silicate. Nodular "granulomas" are scattered throughout the bone marrow. × 10.

FIG. 14.—Rabbit 5. Photograph of the right humerus, the upper part of which is replaced by a large partly-cystic tumour.

FIG. 15.—A photograph of the right femur and tibia in Rabbit 5. The bone marrow of the lower part of the femur is replaced by tumour tissue, and the upper part of the tibia is expanded by a bulky tumour. The marrow cavity of the lower part of the tibia is occupied by fibrous tissue.

FIG. 16.—Thoracic viscera of Rabbit 5, showing numerous blood-borne pulmonary metastases and bulky masses of tumour tissue in the mediastinal lymph-nodes.

FIG. 17.—Showing the structure of the cartilaginous part of the tumour illustrated in Fig. 15 and 10. The tumour tissue is extending into the adjacent muscle, fibres of which are seen in the upper part of the figure. × 45.

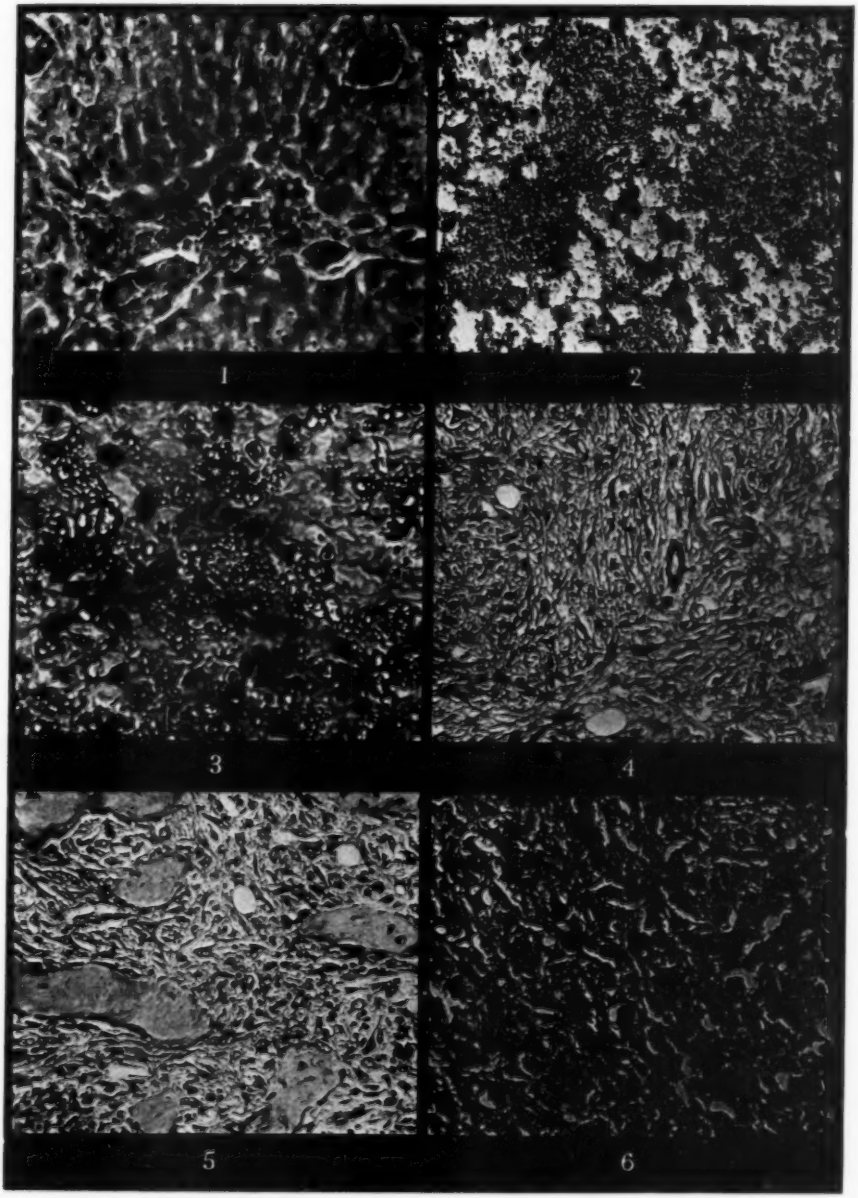
FIG. 18.—The histological structure of the tumour illustrated in Fig. 8. Spaces between normal bone trabeculae are completely occupied by tumour tissue showing specifically bony differentiation of its intercellular matrix. × 85.

FIG. 19.—Showing the histological structure of the bony tumour of the scapula in Rabbit 5. The normal cortex of the bone is to the left, but the entire marrow space of this part of the bone is replaced by tumour tissue. × 40.

FIG. 20.—Invasion of muscle adjacent to bone by spindle-celled tumour tissue. From the tumour of the left femur in Rabbit 10. × 40.

FIG. 21.—An early metastasis in lung tissue. From Rabbit 5. × 40.

FIG. 22.—A metastasis in the liver. From Rabbit 11. × 50.



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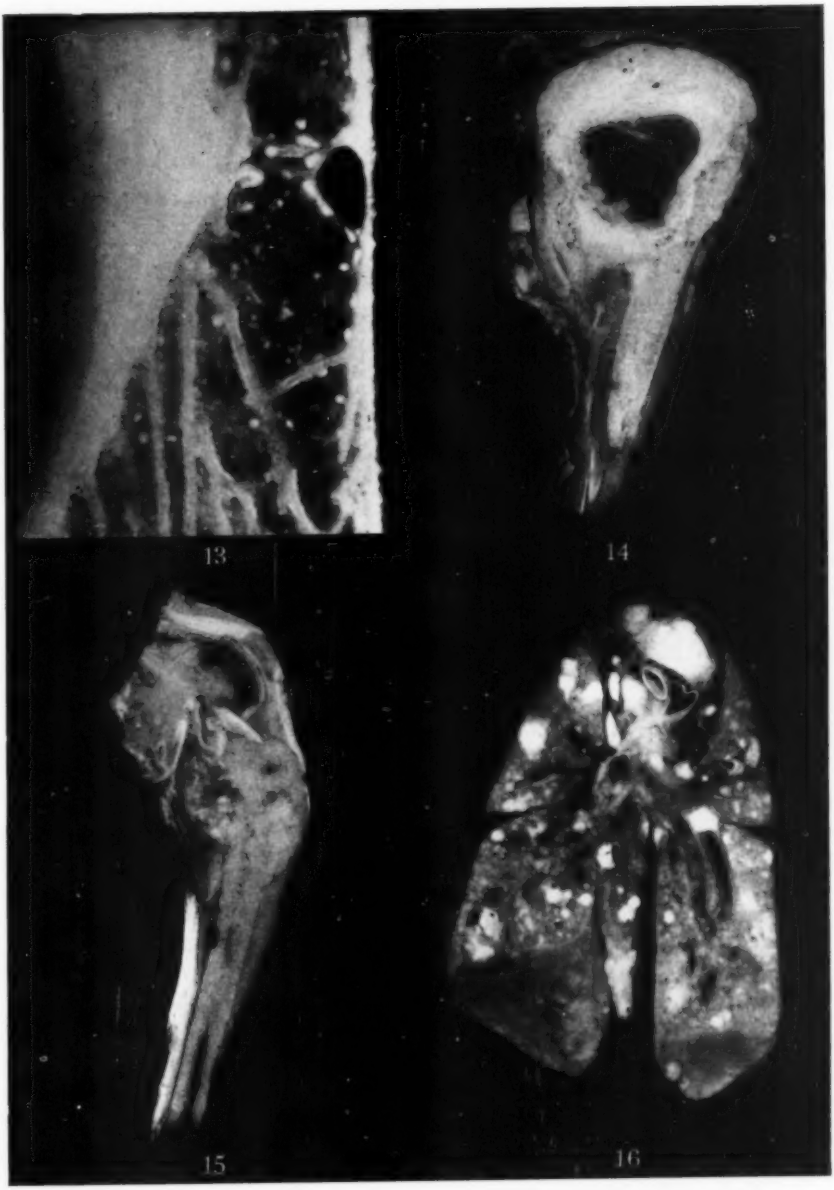

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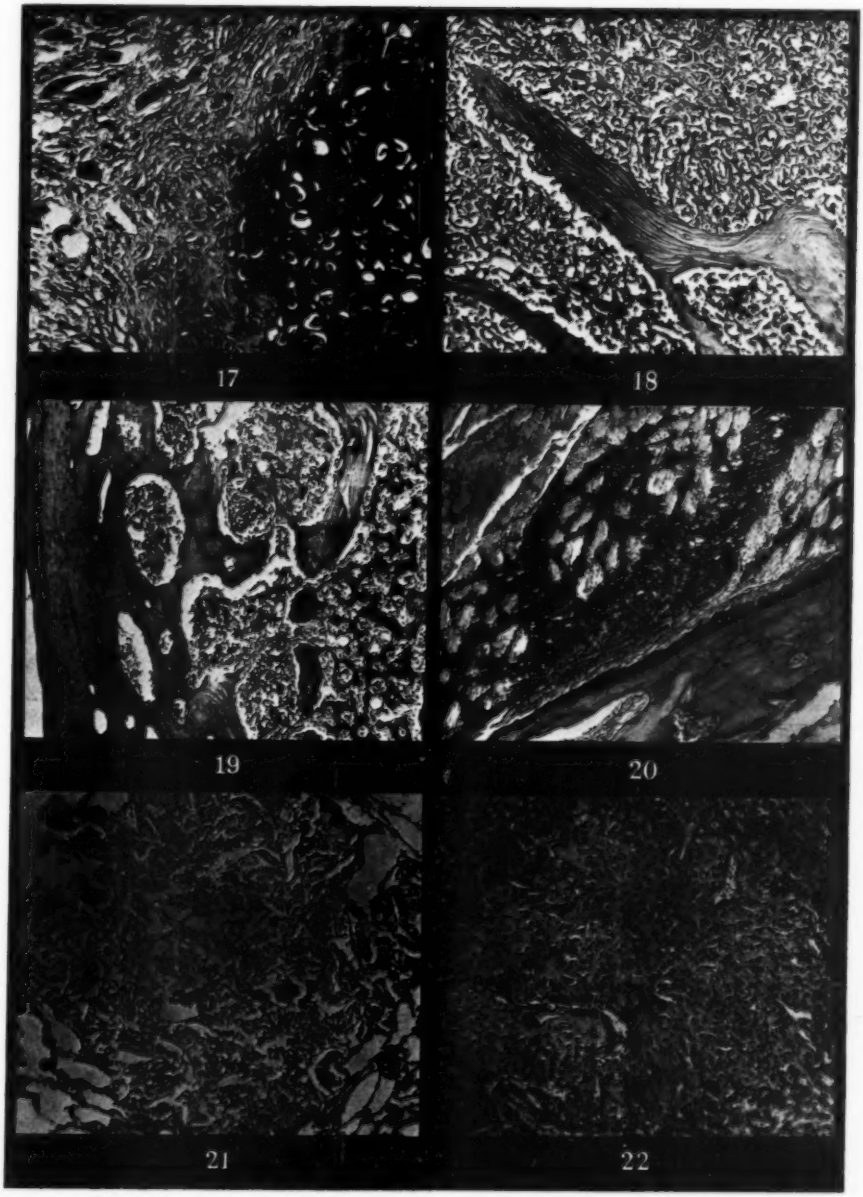

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Barnes, Denz and Sissons.

cartilaginous differentiation, but occasional regions of periosteal bone formation are present, and metaplastic cartilage produced by the displaced periosteum is included in some areas of tumour tissue.

Two small separate tumour nodules are present in the right scapula. One consists of spindle-celled fibrous tissue, while the other is a uniformly bony lesion, whose regular trabeculated pattern is shown in Fig. 19.

Each femur presented radiographic evidence of extensive medullary bone-formation. Histological examination revealed general fibrosis of the bone marrow, with scattered areas of bone-formation. Numerous bulky masses of beryllium-containing phagocytes were present in this tissue. In the lower part of each femur the marrow is completely replaced by a mass of bone-forming tumour tissue, which has begun to extend into the Haversian canals of the dense cortical bone. However, little erosion of cortical bone has been produced in this way, and tumour growth has not yet altered the external contour of the bones.

The tumour at the upper end of the right tibia is seen in Fig. 15, while Fig. 10 is the radiograph of the same lesion. This tumour consists partly of cartilaginous tissue (Fig. 17), partly of osteoid and bony tissue, and partly of undifferentiated spindle-celled tissue. The bone marrow in the lower part of the tibia is fibrosed, and contains areas of medullary bone formation. Aggregates of phagocytic cells loaded with silicate particles are present.

Rabbit 10.

At autopsy this animal presented a huge tumour of the left femur. Ossifying metastases were present in pelvic and abdominal lymph nodes, and in lungs and liver. Smaller tumours were found in the right humerus and the left tibia, and extreme medullary bone formation was present in the remaining long bones.

In radiographs the lesion of the left femur (Fig. 8) is densely ossified, and the greater part of the tumour tissue consists of calcified bone trabeculae. Histologically the tumour is a typical ossifying bone sarcoma, as illustrated in Fig. 18. Adjacent muscle is infiltrated by anaplastic spindle-celled tumour tissue (Fig. 20).

The tumour of the humerus is also a sclerosing one, consisting of anaplastic spindle-celled tissue with scattered areas of osteoid and bony differentiation.

The structure of the tumour of the left tibia is more varied. Some areas consist entirely of cartilaginous tissue, others show extensive osteoid and bony differentiation, while in some places the two tissues merge into a composite "osteochondroid" pattern. Undifferentiated spindle-celled tissue is also present. This varying histological structure is reflected in the radiographic appearance of the lesion, and in Fig. 11 areas of tumour bone formation and of calcification in cartilage can both be seen.

Sections of other long bones, where medullary bone formation had been identified radiologically, showed complete replacement of bone marrow by tumour tissue. In several situations this was beginning to invade the dense cortex of the bone concerned, and to expand the covering periosteum.

In this animal the body of one lumbar vertebra contained a small rounded area of calcifying spindle-celled tumour tissue, 2 mm. in diameter, sharply demarcated from the surrounding normal marrow. This was regarded as a metastasis rather than an independent primary tumour.

Rabbit 11.

At autopsy a tumour of the upper part of the right tibia was present. Histologically this is a typical ossifying bone sarcoma showing uniform and conspicuous bone formation. Ossifying metastases were present in the lungs.

The shaft of the tibia remote from the tumour shows the same fibrous replacement described in Rabbit 4. Again, there is extensive development of bone-trabeculae in the fibrous tissue. Here, however, all stages of transition between fibrous tissue, developing bone trabeculae and malignant bone-forming tumour tissue are present in the same lesion. This suggests that a continuous process is concerned in the development of frankly malignant tissue from the earlier pre-invasive lesion seen, for example, in the humerus of Rabbit 4.

In this animal other parts of the skeleton were not studied in detail.

Rabbit 13.

At autopsy a tumour of the upper part of the right tibia was present, but no metastases were found. Histologically the tumour shows marked structural variation, bony, cartilaginous and anaplastic undifferentiated tissue being present.

The bone marrow of the right femur shows numerous focal aggregates of macrophages loaded with beryllium silicate particles. Radiographs of this animal at the time of death showed early medullary bone formation in each humerus and in the left tibia and femur. These bones were not studied further.

Release of Beryllium in Tissues.

Soluble salts of beryllium have been shown by Aldridge, Barnes and Denz (1949) to be extremely toxic. When injected subcutaneously the beryllium becomes fixed to tissue proteins and remains *in situ* to produce chronic granulomatous lesions.

The beryllium silicates are so insoluble, even in strong acids, that they cannot be brought into solution for estimation by ordinary chemical analysis. But chemical analysis shows that some free beryllium ions are present in aqueous suspensions of beryllium silicates. One mg. of insoluble beryllium silicate contains 5 μg. of soluble beryllium, and similarly 1 mg. of insoluble zinc beryllium silicate contains 130 μg. of soluble beryllium. Treatment of the refractory beryllium silicates with N/100 hydrochloric acid produces a 10 to 20-fold increase in the proportion of soluble beryllium in the aqueous suspension. In order to investigate the release of beryllium from refractory silicates a series of intradermal injections of 0·1 ml. of a 10 per cent. suspension of zinc beryllium silicate was given to a rabbit. At monthly intervals a piece of skin including the injection site was removed by biopsy and histological sections examined. A chronic inflammatory lesion develops around the silicate particles that have been taken up by macrophages. These lesions showed little resolution in the 7-month observation period. When stained by the method developed by Denz (1949) for the histochemical detection of beryllium these sections showed that some soluble beryllium had left the necrotic area and stained the surrounding fibrous tissue faintly and diffusely. This is evidence that soluble beryllium is liberated from the refractory beryllium silicates when in contact with tissues.

DISCUSSION.

These tumours are of interest because of their similarity to human bone sarcomas. Tumours of the same type have been produced in rabbits by radium (Ross, 1936). The range of structural variation in the human bone sarcomas and in those induced by beryllium in the rabbit is apparently identical. Among the experimental tumours some show extensive sclerosis and bone formation, others show cartilagincus differentiation, while a few are anaplastic masses with marked osteolytic propensities. But, just as in human tumours, it is not unusual for several or all of these different structural patterns to be found within a single lesion.

In one respect these tumours differ in their behaviour from their human counterparts. In addition to metastasing by the blood stream, local lymph-node deposits were found in 3 of the 4 animals with tumour dissemination. This frequency of lymph node metastasis is far higher than that occurring in human cases.

The material available from the animals in this experiment made it possible to trace the development of these tumous within the affected bones. While the fully developed tumours present a very different appearance from the medullary bone formation that has been described as an early stage of malignancy, all stages of transition between the two can be traced. It is suggested that these indicate a continuous process of transformation from the early medullary fibrosis to the later obviously malignant lesions, but this is only the interpretation of a number of morbid anatomical observations. Supporting this belief is the occurrence of malignant tissue within the medulla of long bones, while the cortex remains relatively unaffected.

The relationship between the small nodules which are the first change seen within the bone marrow and the subsequent fibrosis and malignant change is uncertain. The nodules produced by the silicates containing beryllium and those produced by the zinc silicate are indistinguishable by the usual histological methods. It seems clear, however, that it is the presence of the beryllium within the nodule that is in some way responsible for the progressive changes that lead eventually to malignancy. Although the beryllium silicates are considered to be insoluble, the observations on material injected intradermally into a rabbit showed that beryllium can be liberated from the particles and can diffuse into the surrounding tissues. Experience of the behaviour of both soluble and insoluble beryllium compounds leads to the conclusion that its toxic action is exerted locally at the site of inoculation or deposition.

Therefore, if beryllium is to be held directly responsible for the tumours, it is much more likely that this beryllium is liberated from deposits in the marrow rather than derived from more distant deposits. The possibility that the silicate may play some part in the development of the tumours is excluded by the fact that Gardner and Heslington (1946) produced tumours with beryllium oxide, and more recently Barnes (1950) has reported that two rabbits that received intravenous injections of a suspension of beryllium metal particles have developed characteristic sarcomas.

It is also apparent that the tissues themselves must contribute a factor, because similar nodules in the liver and lungs arouse very little fibrous tissue reaction, while in the spleen fibrosis and atrophy may go to extreme lengths without showing evidence of malignant change.

SUMMARY.

Bone sarcomata in rabbits developed after a course of intravenous injections of suspensions of zinc beryllium silicate and beryllium silicate, but not after zinc silicate.

Of 17 rabbits surviving the course of injections of zinc beryllium silicate 6 developed tumours ; of 11 survivors of the beryllium silicate injections 1 developed tumours.

Tumours were found between 39 and 83 weeks after the course of injections.

The bone tumours showed a great range of structural variation. The origin of the tumours was multicentric. Malignant blood-borne metastases were common.

The beryllium was considered to be causative. The injected materials were not radioactive.

Since this paper was submitted one further rabbit has died with a sarcoma of the pelvis 30 months after the last of a series of injections of zinc beryllium silicate.

The materials used in these experiments were supplied to us by the Research Laboratories of the General Electric Company, Wembley, to whom we would like to express our thanks.

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THE EFFECT OF RADIOACTIVE IODINE ALONE AND IN COMBINATION WITH METHYLTHIOURACIL AND ACETYLAMINOFLUORENE UPON TUMOUR PRODUCTION IN THE RAT'S THYROID GLAND.

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THE object of the following experiments was an attempt to determine whether radioactive iodine, I^{131}, is carcinogenic to the rat's thyroid gland. It seemed likely that I^{131} would prove so since it is specifically concentrated in the thyroid, whose cells are thereby submitted to a course of β-ray irradiation. The problem is important, since we do not yet know the carcinogenic hazard of I^{131} in clinical medicine, where it is being increasingly used in both tracer and therapeutic doses. Hyperplasia and adenomas of the thyroid have been produced by goitrogens (Griesbach, Kennedy and Purves, 1945), and by goitrogens together with the carcinogen acetylaminofluorene (A.A.F.) (Bielschowsky, 1944). Both treatments have produced malignant tumours (Purves and Griesbach, 1946), the addition of A.A.F. enhancing this effect (Bielschowsky, 1945). Part of the mechanism of tumour production in those experiments was the thyroid hyperplasia induced by the goitrogens. When the following work was started in February, 1948, there were discrepancies in the literature as to the incidence of thyroid tumours in untreated rats and the carcinogenic potencies of goitrogens, which were due to the different rat strains, ages and living conditions, times of application of the drugs and criteria of malignancy used. In view of the discrepancies and in order to compare the action of I^{131} with A.A.F. on normal and hyperplastic thyroids, the rats were divided into 8 groups, treated as follows:

(1) Controls. (2) I^{131}. (3) Methylthiouracil. (4) Methylthiouracil + I^{131}. (5) A.A.F. (6) A.A.F. + I^{131}. (7) A.A.F. + methylthiouracil. (8) A.A.F. + methylthiouracil + I^{131}.

MATERIAL AND METHODS.

A total of 113 hooded rats of the Lister strain were used, pen-inbred from one male and two females. They were fed on " Research (Rat) Cubes " from Heygate & Sons, Northampton, with additional greens. The 2-methyl-4-thiouracil was given as a saturated solution in the drinking tap-water. The 2-acetylaminofluorene was given as a suspension, 1 mg. per 10 ml. of a 0·75 per cent gum-acacia solution in the drinking tap-water. The suspension was made by adding an acetone solution of the aminofluorene to the gum-acacia solution in a beaker and allowing the acetone to evaporate off overnight. The combined drugs were given as a saturated solution of methylthiouracil in the aminofluorene suspension. The rats drank about 8 to 12 ml. fluids per day. The radioactive iodine I^{131} was

carrier-free and given in a dosage of 16 μC., by intraperitoneal injection in 1 ml. of water. This dose is 10 μC. as received from Oak Ridge in 1948, which must be regarded as 16 μC. in accordance with an announcement from Oak Ridge in Spring, 1949.

The experiment was started with 20 rats, which were added to during the following 4 months. The animals were arranged in the groups as described, subdivided into sexes. Halfway through the experiments they were given a six weeks, "summer holiday" off all drugs. The radioactive iodine was injected 48 hours before starting the thiouracil, to allow iodide accumulation into the thyroid gland. The I^{131} injection was repeated after the "summer holiday," so that the total dosage of radioactive iodine was 32 μC. per rat, given in 2 doses of 16 μC. separated by a 5½ months' interval. The rats were killed by coal gas at an average age of 15 months, having had treatment for an average of 13 months (including the rest period). All animals, whether they had died or been killed, were autopsied. The trachea and thyroid attached was fixed in Helly's fluid; the thyroid was then dissected off the trachea and weighed to the nearest milligram. The thyroids were embedded in wax, serially cut at 5μ and mounted on to slides as ribbons of 8 to 14 sections, including both lobes. Every fourth slide was stained by haemalum and eosin. Sections were also taken from the lungs, of a number of pituitaries and of all macroscopically abnormal organs. The number of rats used was 98 to obtain data for the main experiment, at the end of which the remaining 15 from various groups were put aside for study of the thyroid I^{131} uptake by direct measurement and autoradiography. The autoradiographs were made by the stripping film technique described in detail by Doniach and Pelc (1950), the rats being injected with 16 μC. carrier-free I^{131} 24 hours before being killed.

RESULTS.

The indication of adenomas by plus signs in Tables I, II, III, IV and V is not devised to be any more than a crude indication of tumour incidence; the object was to seek for major rather than minor differences resulting from the various treatments of these small numbers of rats. I have not given a detailed description of the histology and histogenesis of the adenomas, because in addition to the references cited in the introduction, there have appeared further detailed illustrated descriptions by Purves and Griesbach (1947), Money and Rawson (1947), Laqueur (1949), and Hall and Bielschowsky (1949). Autopsies on the majority of the animals found dead revealed no more than wasting and post-mortem autolysis; pathological findings are recorded in the text.

Controls (Table I).

The thyroid sections in general showed large peripheral follicles, rich in deeply eosinophilic colloid, lined by a cuboidal epithelium and smaller central follicles containing a paler, finely vacuolated colloid lined by a slightly higher cuboidal epithelium. In each of 4 rats a sharply demarcated single or sometimes double spherical focus was found 50 to 100 μ across of closely packed spheroidal cells with voluminous eosinophilic cytoplasm and vesicular nuclei, showing an occasional mitosis (Fig. 1). These were seen to be solid on serial section and surrounded by a delicate reticulin fibre capsule on silver stain. They appeared to have arisen by dedifferentiation and proliferation of the lining cells of one or two

contiguous follicles and were regarded as solid adenomas. One adenoma, Female Rat 3, showed focal differentiation of newly-formed microfollicles. Solid adenomas were found more frequently in the treated rats, where some showed a tendency to a trabecular arrangement of their constituent cells.

TABLE I.—*Controls.*

Rat.	Sex.	Age (months).	Mode of death.	Body weight (g.).	Thyroid weight (mg.).	Thyroid adenomas.
1	Male	12	Died	135	10	*Nil*
2	,,	13	Killed	305	20	,,
3	,,	13	,,	305	19	,,
4	,,	13	,,	300	19	,,
5	,,	13	,,	300	24	,,
6	,,	13	,,	340	21	+
7	,,	13	,,	295	20	+
8	,,	17	,,	360	32	*Nil*
1	Female	14	,,	180	12	,,
2	,,	14	,,	210	13	,,
3	,,	14	,,	200	12	+
4	,,	14	,,	170	10	*Nil*
5	,,	14	,,	200	12	,,
6	,,	17	,,	255	19	+
7	,,	17	,,	250	23	*Nil*

+ Represents the finding of 1 adenoma per gland.

Radioactive Iodine (*Table II*).

The body weights were normal, but the thyroid glands were only half the weights of the controls as shown in Table VI. The thyroid tissue in general differed from the controls in showing a greater variation in cell height, follicle size and colloid storage. Adenomas were present in at least 10 out of the 16 rats. They were mostly follicular in type, consisting of foci of colloid containing

TABLE II.—*Radioactive Iodine.*

Rat.	Sex.	Age (months).	Treatment* (months).	Mode of death.	Body weight (g.).	Thyroid weight. (mg.).	Thyroid adenomas.
1	Male	14	12	Died	250	9	*Nil*
2	,,	15	13	Killed	370	7	++
3	,,	15	13	,,	365	8	+++
4	,,	16	14	,,	350	11	++
5	,,	16	14	,,	340	8	+++
6	,,	16	14	,,	335	11	+++
1	Female	16½	14	,,	230	10	*Nil*
2	,,	16½	14	,,	255	12	,,
3	,,	16½	14	,,	230	11	,,
4	,,	16½	14	,,	250	7	++
5	,,	16½	14	,,	240	6	*Nil*
6	,,	16½	14	,,	250	5	+
7	,,	17	14½	,,	120	5	+
8	,,	17	14½	,,	240	10	+++
9	,,	17	14½	,,	240	7	+++
10	,,	18	15½	,,	220	13	*Nil*

* Number of months before the rats were killed when they received the first of their two injections of radioactive iodine.

+ Represents 1 adenoma per gland; ++, 2 or 3 adenomas; +++, multiple adenomas.

follicles and tubules lined by closely packed cuboidal and low columnar cells with small darkly staining nuclei and basophilic cytoplasm (Fig. 2). In many areas it was difficult to differentiate the adenomas, and the number of plus signs awarded in the thyroid adenoma column of Table II is probably an underestimate. The pre-adenomatous foci (Fig. 2) were similar to those described by Laqueur (1949), consisting of a few follicles larger than their neighbours lined by closely packed cells containing hyperchromatic nuclei. Female Rat No. 5 showed a parathyroid adenoma; her bones were not sectioned. At autopsy Male Rat No. 1 showed cirrhosis of the liver and a malignant lymphoma massively involving both the abdominal and thoracic lymph-nodes.

Methylthiouracil (Table III).

The rats were lively and appeared healthy, but they only developed to half the body weight of the controls. Their thyroid glands (Table VI) were five times heavier than those of the controls. Sections showed a remarkable follicular hyperplasia, gross vascularity and almost total colloid depletion. The follicular epithelium was columnar and higher in the central follicles, whose lumens were thereby smaller than those of the peripheral follicles. Most glands showed focal fibrous thickening of the capsule, which often traversed the parenchyma and enclosed thyroid follicles, giving rise to a spurious appearance of infiltration by parenchymatous tissue and of adenoma formation (Fig. 3). In addition, the very close proximity of some follicles to large thin-walled subcapsular venous

TABLE III.—*Methylthiouracil.*

Rat.	Sex.	Age (months).	Treatment (months).	Mode of death	Body weight (g.).	Thyroid weight. (mg.).	Thyroid adenomas.
1	Male	11	9	Died	120	46	*Nil*
2	„	12	11	Killed	130	72	+
3	„	13	12	„	170	114	*Nil*
4	„	13	12	„	160	127	++
5	„	13	12	„	170	114	*Nil*
6	„	13	11½	Died	105	85	„
7	„	16	14	Killed	170	107	++
8	„	16	14	„	160	106	*Nil*
9	„	16	14	„	160	99	+
1	Female	12	10	„	100	29	*Nil*
2	„	16	15	„	155	100	+
3	„	16	15	„	125	120	+
4	„	16	15	„	135	99	+
5	„	16	15	„	85	53	++
6	„	16	15	„	115	76	+
7	„	16	15	„	125	102	++
			Methylthiouracil + Radioactive Iodine.				
1	Female	14½	12	Died	150	..	Thyroid lost
2	„	16½	14	Killed	135	45	+++
3	„	16½	14	„	125	44	+++
4	„	16½	14	„	120	364	++++ cancer
5	„	16½	14	„	115	8	+++

+, ++, +++ as in Table II.
++++ Represents adenomatous replacement of most of the thyroid gland.

inusoids mimicked a pre-invasive change (Fig. 3). However, the cells of these extra and intracapsular and juxta-venous follicles were quite innocent in appearance, and in no way different from those constituting the main mass of the glands. Many arteries showed a remarkable muscular hyperplasia of their walls and reduction of their lumens. The pericapsular tissue of a few glands was inflamed and infiltrated with polymorphs. Solid and follicular adenomas, mostly the latter, were present in 10 of the 16 glands. Some of the adenomatous follicles were grossly distended; a few contained eosinophilic colloid. Many of the adenomas were grouped around a large blood vessel (Fig. 4). At autopsy Male Rat No. 6 showed massive suppuration of the peri-urethral glands.

Methylthiouracil and radioactive iodine (Table III).

The body weights of these 5 rats did not differ significantly from those treated with methylthiouracil alone. The thyroid glands were smaller except for the cancerous one, though larger than those of untreated controls (Table VI). The thyroid of Rat No. 1, which was lost, was noted at autopsy to be only moderately enlarged. The thyroids of Rats 2, 3 and 5 showed a typical thiouracil follicular hyperplasia and colloid depletion of surviving thyroid tissue. Most of the tissue, however, was replaced by very numerous adenomas, solid, mixed solid and follicular, follicular, and papilliferous cystic (Fig. 5). Many were rich in eosinophilic colloid. There were areas present of an appearance suggestive of pre- or early adenomatous change, difficult to differentiate from adenomas proper. The effect of the I^{131} was such that every one of the glands in this admittedly small group showed a most striking increase in adenoma formation as compared with the rats treated with methylthiouracil alone. Both lobes of the enormously enlarged thyroid of Rat No. 4 were almost entirely replaced by a fantastic mixture of all varieties of large adenomas, some of them very cellular. Many veins outside the gland were plugged with mixed solid and microfollicular tumour (Fig. 6). Growth of the solid adenoma type was present in random pulmonary arteries (Fig. 7), and one deposit of similar tumour had enlarged and mostly replaced one of the adrenal glands. This rat was regarded as having developed a cancer of the thyroid.

Acetylaminofluorene (Table IV).

The body weights were within normal limits. The thyroid weights varied, as did the associated histology. Most of them appeared similar to controls, but the thyroids of Male Rat No. 6 and Female Rats Nos. 6, 7 and 8 showed a definite moderate hyperplasia and colloid depletion. Adenomas were present in 7 of the 14 rats, a few were follicular colloid containing (Fig. 8), most were solid with early follicle formation. It was difficult either to diagnose or rule out the presence of adenomas in the somewhat autolysed thyroids of the 4 rats which had died.

Acetylaminofluorene and radioactive iodine (Table IV).

Of the 6 rats 5 were wasted and died. Rats 3 and 5 showed massive suppuration of the peri-urethral glands. Autolysis rendered the histological diagnosis of adenomas difficult. They were mostly large colloid secreting, follicular in type (Fig. 9).

TABLE IV.—*Acetylaminofluorene.*

Rat.	Sex.	Age (months).	Treatment (months).	Mode of death.	Body weight (g.).	Thyroid weight (mg.).	Thyroid adenomas.
1	Male	6½	4	Died	110	..	*Nil*
2	„	8	7	„	110	..	„
3	„	10	9	„	170	17	„
4	„	15	14	Killed	300	26	+
5	„	15	14	„	270	26	*Nil*
6	„	17½	14½	„	360	44	+
1	Female	12	11	„	145	10	++
2	„	15	14	Died	120	13	*Nil*
3	„	15	14	Killed	185	16	„
4	„	15	14	„	180	15	+
5	„	15	14	„	195	15	+
6	„	17	15	„	250	26	++
7	„	17	15	„	250	24	++
8	„	17	15	„	225	21	*Nil*
		Acetylaminofluorene + Radioactive Iodine.					
1	Male	12	9½	Died	160	9	*Nil*
2	„	15	12½	„	190	12	++
3	„	14½	12	„	250	12	*Nil*
4	„	15½	13	„	200	12	++
5	„	16	13½	„	180	8	*Nil*
6	„	16½	14	Killed	300	8	++

+, ++ as in Table II.

Acetylaminofluorene and methylthiouracil (Table V).

The body weights and thyroid weights were comparable to those of rats treated with methylthiouracil alone. The general thyroid histology also closely resembled that of the methylthiouracil treated rats showing hyperplasia, colloid depletion, thickened capsules and vessels. Pre-adenomatous hyperplasia was a little more marked. The thyroids of all the rats treated for 10 months or more abounded in multiple medium-sized adenomas, solid, follicular and mixed (Fig. 10). Female Rats 1 and 2 died of widespread involvement of thoracic and abdominal lymph-nodes by malignant lymphoma. Male Rat 1 showed kidney suppuration and Male Rat 2 suppuration of the peri-urethral glands.

Acetylaminofluorene, methylthiouracil and radioactive iodine (Table V).

The thyroid weights (excluding Rat No. 6) were a third of those of the previous group, but still three times heavier than those of rats treated with I^{131} alone (Table VI). All contained multiple large adenomas, which in Rats 2, 3, 4, 5 and 6 had almost totally replaced normal thyroid tissue. They were similar in appearance to those seen in the rats treated with methylthiouracil and I^{131}. Rat No. 6, whose thyroid was three times heavier than the rest of the group, showed plugs of tumour cells of the solid adenoma type within the lumen of veins outside the thyroid gland (Fig. 11). Random lung sections were free of growth. This rat was regarded as having developed a cancer of the thyroid. Rat No. 3 died with massive suppuration of the peri-urethral glands.

The results of the studies on the 15 supplementary rats will not be detailed in this paper, except that one autoradiograph, considered relevant to the discussion, is included in the illustrations (Fig. 12).

TABLE V.—*Acetylaminofluorene + Methylthiouracil.*

Rat.	Sex.	Age (months).	Treatment (months).	Mode of death.	Body weight (g.).	Thyroid weight (m.g.).	Thyroid adenomas.
1	Male	9	7½	Died	110	100	*Nil*
2	„	10½	8	„	120	60	„
3	„	14	11½	Killed	150	83	+++
4	„	14½	12	Died	130	67	++
5	„	14½	13	Killed	165	77	+++
6	„	14½	13	„	185	168	+++
7	„	14½	13	„	165	100	+++
8	„	15	12½	„	200	129	+++
9	„	15	12½	„	180	136	+++
10	„	17½	15	„	150	116	+++
1	Female	11½	10	Died	120	60	+++
2	„	11½	10	„	..	117	+++
3	„	14½	13	Killed	160	119	+++
4	„	14½	13	„	125	125	++++
5	„	14½	13	„	130	120	+++
6	„	15	13	Died	110	108	+++
7	„	15	13	Killed	125	124	+++
8	„	15	13	„	130	85	+++
9	„	15	13	„	155	107	++++
10	„	15½	11½	Died	110	79	+++
	Acetylaminofluorene + Methylthiouracil + Radioactive Iodine.						
1	Male	14	12	Died	120	40	+++
2	„	14½	11½	„	130	36	++++
3	„	14½	12	„	125	27	++++
4	„	14½	12	Killed	140	36	++++
5	„	15	13	Died	130	27	++++
6	„	16	14	Killed	175	99	++++ cancer

+, ++, +++, ++++ as in Tables II and III.

DISCUSSION.

The above results show that radioactive iodine increased the incidence of thyroid adenomas in all groups except the A.A.F., which will be discussed later. The adenomas were larger in size as well as increased in number, and showed evidence of malignancy by a gross increase in size and by dissemination outside the thyroid in two instances. The assessment of radiation dosage administered to the thyroid by I^{131} is a difficult one. In the first place the percentage uptake varies with the iodine content of the diet and the surrounding temperature. We have found iodine uptake by the thyroid 24 hours after administering I^{131} varying from 10 per cent in hot weather to 30 per cent when it was cool. Secondly, as shown by autoradiography (Leblond and Gross, 1948; Doniach and Pelc, 1949), there is a wide variation in concentration from follicle to follicle; the peripheral follicles in the rat take up considerably less iodine than the central ones. This is only partly balanced by the more rapid disappearance of iodine from the central follicles. The radiation effects are due mostly to β radiation, since most of the γ rays, which are much more penetrating, will not be absorbed in the small thyroid of the rat. This uneven distribution of I^{131} almost certainly accounts for the remarkable resistance of the thyroid to radiation as computed in roentgen equivalents. The associated variation in cell activity may similarly account for the comparative resistance to X-irradiation. Destruction of active cells is pre-

sumably followed by a regenerative activity of surviving resting cells, and the gland will function normally. Feller, Chaikoff, Taurog and Jones (1949) have recently estimated that after injection of 30 μC. of I^{131} the rat thyroid receives an overall dose of 28,000 reps (roentgen equivalents physical) during the ensuing 10 days. They found that this radiation did not interfere at all with the ability of the thyroids to take up further iodine, produced no histological changes, and showed no alteration of thyroid function as gauged by various physiological tests. Their rats averaged 40 per cent maximal uptake of I^{131}, ours was nearer 20 per cent, and our total I^{131} injected was 32, theirs was 30 μC., therefore our dosage was roughly 15,000 roentgen equivalents physical. Skanse (1948) tested the effect on the chick thyroid of injections of 1, 10 and 50 μC. of I^{131}. He calculated that the total doses received by the glands were respectively 1700, 13,000 and 60,000 reps. In these young growing animals the 10 μC. I^{131} (13,000 reps) produced a significant inhibition of thyroid gland growth, though not of function, after 16 days. From the long-term view in our experiments this dosage proved damaging, as shown in Table VI, where it can be seen that the thyroid weights of all I^{131} treated rats in all groups was one-half to one-third of their respective controls. Clearly though, enough thyroid had survived to maintain normal growth of the rats, since the body-weights did not differ from their respective controls in spite of the fact that the first dose of I^{131} was administered at an average age of 2½ months. The human thyroid is 1000 times heavier than the

EXPLANATION OF PLATES.

FIG. 1.—Thyroid of Female Control Rat No. 6 showing a " solid " cellular adenoma. × 145.

FIG. 2.—Thyroid of Male I^{131} Rat No. 3 showing a trabecular adenoma below and to the right, a follicular adenoma above and to the left and a parathyroid above and to the right. Between the adenomas are a few pre-adenomatous foci consisting of two or three large follicles lined by closely packed hyperchromatic cells. × 36.

FIG. 3.—Thyroid of male methylthiouracil Rat No. 8 showing a thickened capsule separating groups of follicles from the main gland. The follicles in general are small, hyperplastic and empty of colloid. They are closely apposed to the endothelium of a large subcapsular venous sinusoid in the upper left half of the photomicrograph. One artery, traversing the gland, shows a marked muscular hyperplasia of its wall. Above it lies an elongated parathyroid embedded in the gland. × 40.

FIG. 4.—Thyroid of Male methylthiouracil Rat No. 9 showing follicular hyperplasia, thickening of the capsule and adenoma formation round a large blood vessel. × 38.

FIG. 5.—Thyroid of Female methylthiouracil I^{131} Rat No. 2 showing multiple adenomas of varying morphology in a hyperplastic gland. × 40.

FIG. 6.—Thyroid of Female methylthiouracil I^{131} Rat No. 4 showing adenomatous replacement of the gland, a thickened capsule and a plug of tumour tissue filling a pericapsular vein, above and to the right. × 40.

FIG. 7.—Lung of Female methylthiouracil I^{131} Rat No. 4 showing emboli of tumour cells in branches of the pulmonary artery. × 40.

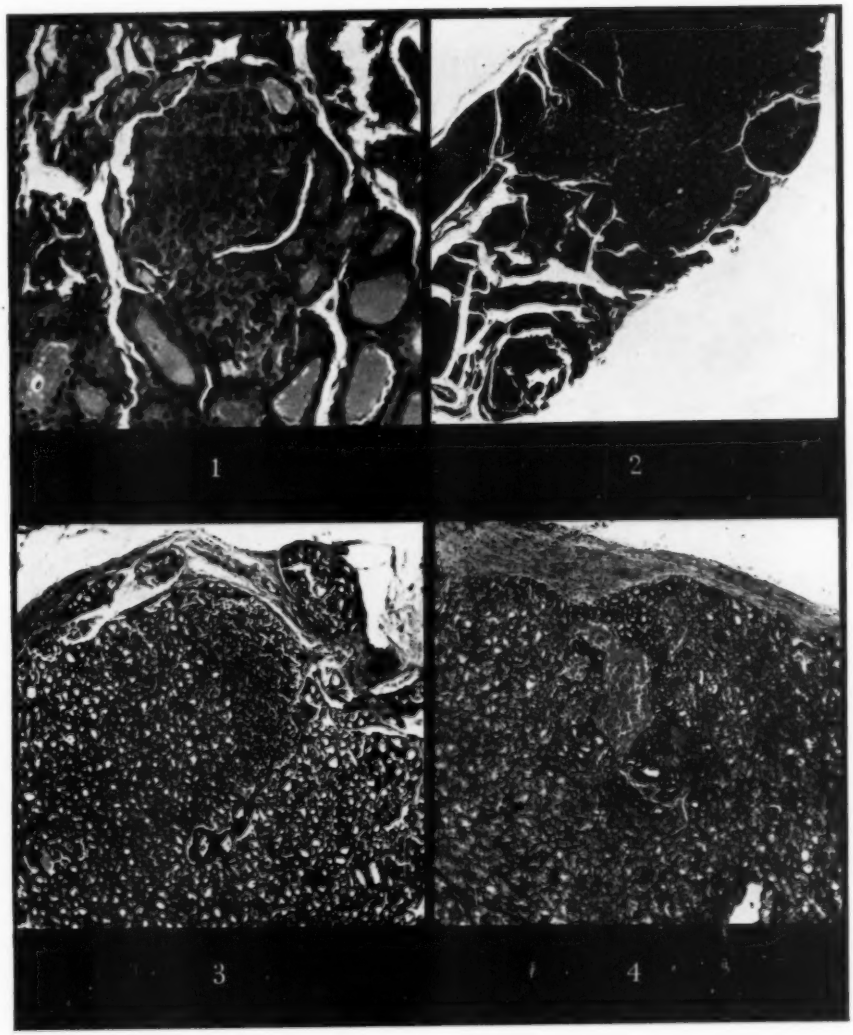
FIG. 8.—Thyroid of Male A.A.F. Rat No. 4 showing a colloid containing follicular adenoma in an otherwise normal gland. × 45.

FIG. 9.—Thyroid of Male A.A.F. I^{131} Rat No. 2 showing a large papillary colloid secreting adenoma in an autolysed gland. × 40.

FIG. 10.—Thyroid of Female A.A.F. methylthiouracil Rat No. 3 showing multiple adenomas in a hyperplastic gland with a thickened capsule. × 40.

FIG. 11.—Thyroid of Male A.A.F. methylthiouracil I^{131} Rat No. 6 showing adenomatous replacement of the gland and a plug of tumour tissue in a neighbouring vein, below and to the left. × 40.

FIG. 12.—Haemalum stained autoradiograph of a 15-month-old female A.A.F. methylthiouracil rat killed 12 days after cessation of 13 months' treatment, showing intense blackening over the colloid of the normal follicles and less intense but definite blackening over the colloid secreted by the adenoma lying in the centre of the photomicrograph. The rat had received 16 μC. of I^{131} 24 hours before being killed. × 40.



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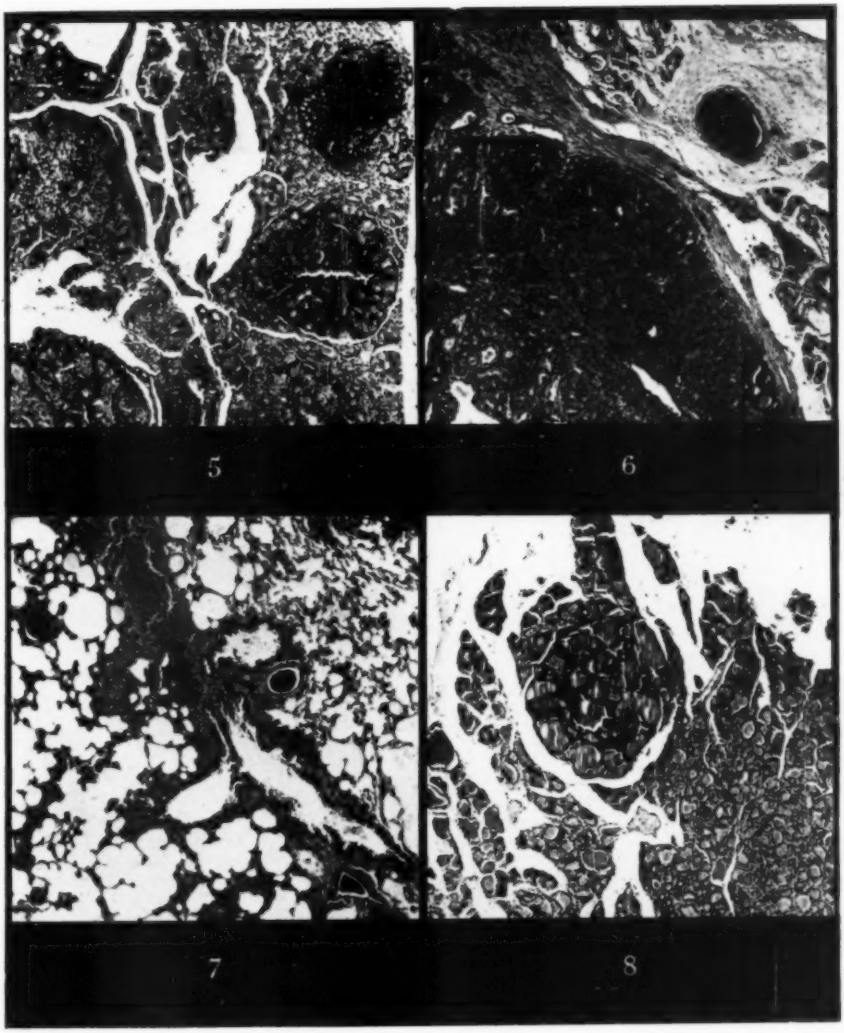

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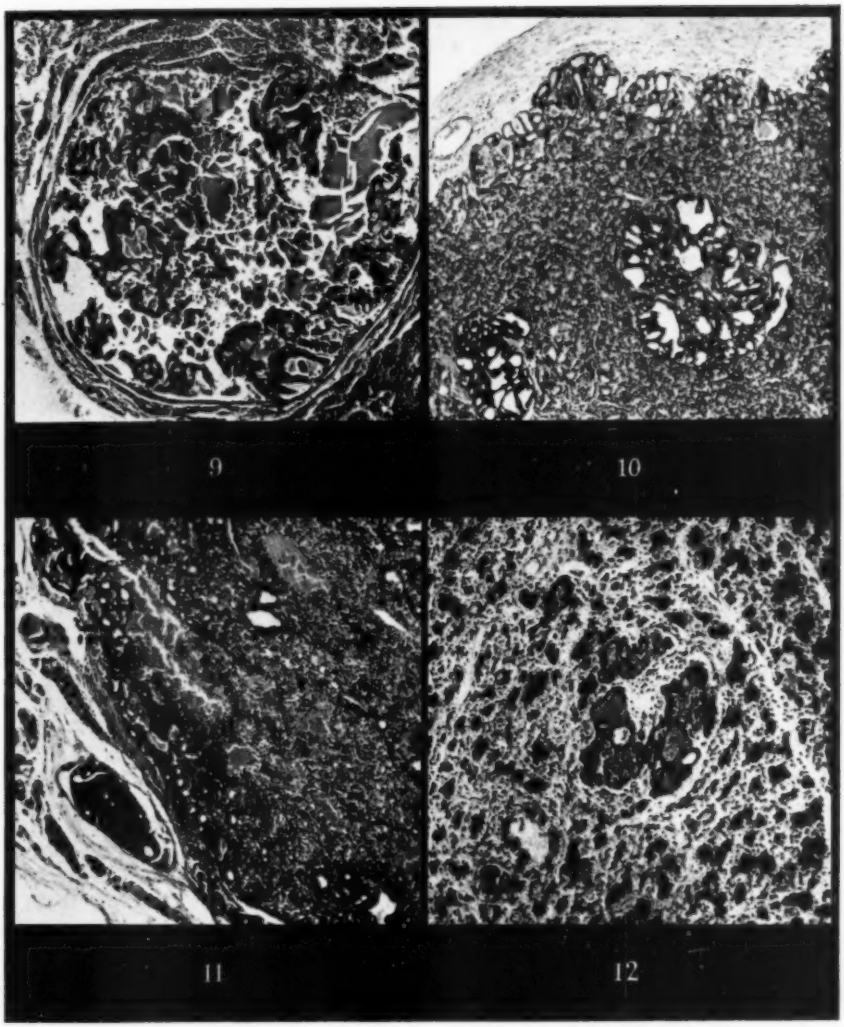

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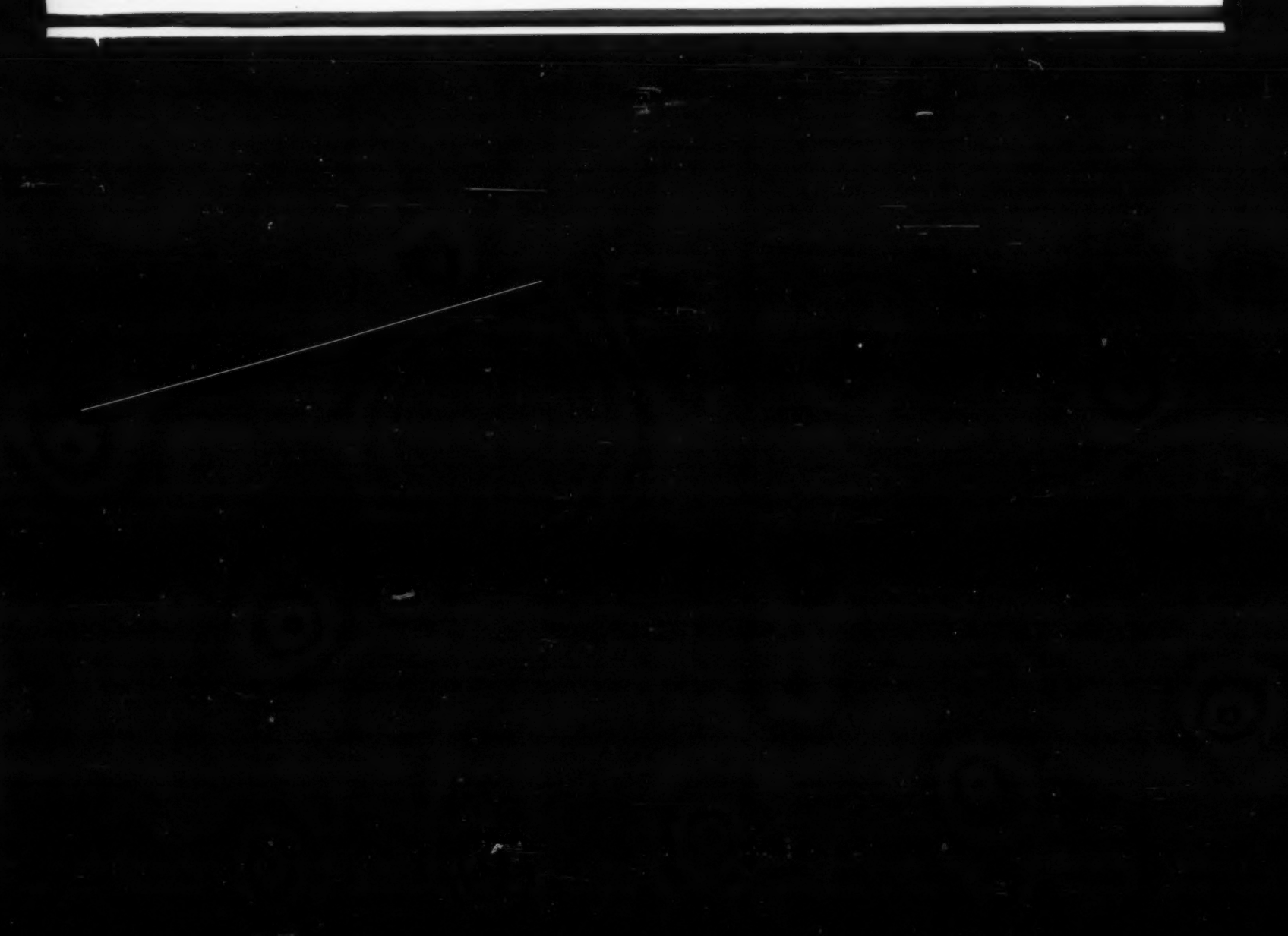

rat's, so that doses of 10 to 20 mC. might be regarded as likely to induce adenomas and possibly occasional cancers in the hyperplastic glands. This is just the order of dosage prescribed for the treatment of thyrotoxicosis!

With regard to the A.A.F. experiments, a dose was sought which would prove carcinogenic to the thyroid, but would not be large enough to kill off the animals prematurely with tumours elsewhere. As can be seen by comparison of Table V with Table III, 1 mg. a day proved satisfactory in strikingly increasing the incidence of adenomas in methylthiouracil treated rats, fully confirming Bielschowsky's (1944, 1945) original observations. At the same time no liver or breast or bladder tumours were found in any of the A.A.F. treated rats. Hall (1948) also observed that 10 to 15 mg. per week failed to produce tumours of other organs. Comparison of A.A.F. treated rats (Table IV) with controls (Table I) shows no striking increase in adenomas, though a larger series might prove significant. Cox, Wilson and De Eds (1947) found primary thyroid tumours in 11 rats out of 84 treated with A.A.F. alone. Nor does comparison of A.A.F. $+I^{131}$ with I^{131} alone (Table II) show an increase in adenomas. However, most of the A.A.F. and I^{131} thyroids were autolysed. But the very large size of the detectable adenomas in the A.A.F. and I^{131} is suggestive that the I^{131} had been effective.

TABLE VI.—*Summary of Thyroid Weights.*

Treatment.	Number of rats.	Sex.	Mean thyroid weight (mg.).
Controls	8	Male	$20 \cdot 6 \pm 5 \cdot 7$
Controls	7	Female	$14 \cdot 4 \pm 4 \cdot 3$
I^{131}	6	Male	$9 \pm 1 \cdot 6$
I^{131}	10	Female	$8 \cdot 6 \pm 2 \cdot 6$
Methylthiouracil	9	Male	$96 \cdot 6 \pm 23 \cdot 7$
"	7	Female	$82 \cdot 7 \pm 29 \cdot 4$
" + I^{131}	3	"	$32 \cdot 3 \pm 17 \cdot 1$*
Acetylaminofluorene	4	Male	$30 \pm 8 \cdot 0$
"	8	Female	$17 \cdot 5 \pm 5 \cdot 2$
" + I^{131}	6	Male	$10 \cdot 1 \pm 1 \cdot 9$
" + thiouracil	10	Male	$103 \cdot 6 \pm 32 \cdot 2$
" + thiouracil	10	Female	$104 \cdot 4 \pm 21 \cdot 0$
" + thiouracil + I^{131}	5	Male	$33 \cdot 2 \pm 5 \cdot 8$†

* Excluding Rat No. 4 (methylthiouracil I^{131}).
† Excluding Rat No. 6 (A.A.F. methylthiouracil I^{131}).

The nature of these adenomas is not easy to assess. Physiological thyroid activity is effected both by follicular hyperplasia and thyroxine output, both under the influence of anterior pituitary thyrotrophic hormone. Thyrotrophic hormone output is regulated by the blood level of thyroxine; a decrease in the

latter is associated with an increase of the former and a resultant follicular hyperplasia. This is the accepted mechanism for the goitrogenic action of antithyroid drugs, such as thiouracil (Astwood, Sullivan, Bissell and Tyslowitz, 1943; Mackenzie and Mackenzie, 1943). The experimentally produced adenomas can be regarded as representing focal (nodular) hyperplasia. Griesbach, Kennedy and Purves (1945) and Purves and Griesbach (1947) found them to respond to increased and decreased thyrotrophic hormone stimulation. The functional nature of these adenomas was confirmed in the present series by autoradiography of a thyroid from an A.A.F. + methylthiouracil-treated rat (Fig. 12), where a follicular adenoma (after 12 days' cessation of drug treatment) was found to take up I^{131}, though less actively than surrounding follicles. Nevertheless, this nodular hyperplasia must be regarded as a definite deviation from the normal, akin to tumour production both on morphology, and on the grounds that it may lead to malignant change as shown by previous and the present work. The position can be regarded hypothetically as follows: The rat thyroid is normally continually submitted to a varying degree of thyrotrophic hormone stimulation. This is associated in control rats with occasional deviations from a diffuse hyperplasia to the formation of abnormal focal nodular hyperplasia. Increased thyrotrophic hormone stimulation by goitrogens (Table III) increases the number of adenomas. Prolonged for 2 years, this alone may lead to malignancy (Purves and Griesbach, 1946). An added carcinogen, A.A.F., markedly increases adenoma production. In the above experiments the effect of added I^{131} appears comparable with added A.A.F. in so far as it increases adenoma production. I^{131} differs from A.A.F. in that it is more effective on its own. This may well be due to the fact that the dosage of I^{131} used was destructive enough to the thyroid temporarily to diminish thyroxine formation, and thus step up thyrotrophic hormone production. It is interesting to note in this connection that the same number of rats showed adenomas in the I^{131} group (Table II) as in the methylthiouracil group (Table III), and that the number of adenomas was greater in the former group. To summarize, the I^{131} acted indirectly as a thyrotrophic hormone stimulant and directly as a carcinogen, both results by virtue of its β-ray emission, the effects varying in the gland according to the state of the cells and the dose received.

The problem remains as to why the controls show an occasional deviation from the normal in the formation of adenomata, and how does prolonged excessive thyroid stimulation by the pituitary intensify this deviation, and by what means additional carcinogen leads to a further increase in adenomas. One obvious role of thyrotrophic hormone is the production of an increased volume of proliferating thyroid tissue. Hall (1948) has compared this action with that of the promoting cocarcinogenic action of croton oil in the induction of skin papillomata. But Hall and Bielschowsky (1949) have recently concluded that though A.A.F. acts as an initiator of neoplastic thyroid cells (hastening the appearance of benign multiple adenomas, it is not essential for the production of malignancy, which can result from continuous prolonged thyrotrophic hormone stimulation alone. Prolonged thyrotrophic hormone stimulation appears, therefore, to have both an initiating and promoting carcinogenic action upon the thyroid gland. It is not surprising that this should summate with the carcinogens A.A.F. and I^{131}. Mottram (1938) produced benign and malignant skin tumours in mice by the combined action of benzpyrene painting, insufficient on its own to produce warts,

together with a single exposure of 800 to 2500 r units of beta radiation. In view of these findings, the safety of I^{131} therapy for thyrotoxicosis must be questioned. However, a dosage sufficient to eliminate the thyroid, provided it is followed by maintenance with thyroxine, would presumably not be carcinogenic. The time factor is important; it is likely that tumour formation would require a much longer time to be revealed in the human than in the rat.

I hestitate to join the vexed discussion of histological diagnosis of thyroid malignancy. But there is no doubt that the accepted criteria for other cancers do not always hold in the case of the thyroid. The frequent use in clinical medicine of the term "adenoma malignum" speaks for itself. Experimentally, Gorbman (1947) found hyperplastic thyroid tissue within the lumens of pulmonary vessels of chronically thiouracil treated mice as well as parenchymatous pulmonary deposits considered to be probably thyroid in origin. Yet a return to normal diet after prolonged goitrogen treatment was followed by involution of the supposedly cancerous thyroids. He considered that the hyperplastic thyroid tissue had entered the thyroid veins mechanically rather than by "malignant infiltration," and that the changes were entirely benign. The diagnostic significance of local venous tumour embolism is indeed difficult to assess in nodular goitres. One may assume, therefore, that two types of metastatic thyroid tissue exist, one, possibly resulting from a mechanical breakaway, still dependent upon thyrotrophic hormone for its survival, the other independent. Nevertheless, from the host's point of view the first type of metastasis may prove just as embarrassing as the second, so long as thyrotrophic hormone stimulation is maintained. And a lethal metastasizing tumour can reasonably be classified as malignant. The metastasizing prostatic tumour caused temporarily to regress by suppression of androgens essential for its maintenance might be regarded as an intermediate type of cancer. Prolonged thyroxine treatment, used in clinical medicine in the therapy of nodular goitre, may, by inhibiting thyrotrophic hormone production, not only shrink the goitre, but lessen the likelihood of mechanical production of thyroid metastases and of their survival.

SUMMARY.

The carcinogenic potency on the thyroid of 32 μC. of I^{131} was tested in a small series of rats alone, in combination with methylthiouracil, with acetylaminofluorene, and with the combined drugs. The radioactive iodine was found to significantly increase the formation of thyroid adenomas as compared with non I^{131} treated controls in the following groups: those treated with I^{131} alone, those treated with methylthiouracil and those treated with methylthiouracil plus acetylaminofluorene. One thyroid cancer was found in each of the latter two groups. The findings are discussed.

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STUDIES ON THE INDUCTION OF LUNG CANCER IN MICE.

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LUNG tissue is unsuitable for direct injection of carcinogens, consequently pulmonary tumours in laboratory animals have hitherto been induced by applying the carcinogen at some remote site, by injecting it subcutaneously, intratracheally, intravascularly, intraperitoneally, or by including it in the diet. These indirect methods are often laborious, and tumour induction may take a considerable time.

Recently the author (Horning, 1947) reported the induction of pulmonary tumours after relatively short periods of treatment by the direct application of carcinogens to adult lung tissue growing as subcutaneous homologous grafts in host animals. Further experiments have shown that success with this technique depends upon several factors, and the object of this paper is to describe additional observations.

TECHNIQUE.

The method of tumour induction consists in isolating small strips of lung from 6–12 weeks Strain A mice, impregnating the grafts with crystals of a carcinogen (2-acetylaminofluorene, 2-aminofluorene, or 20-methylcholanthrene) prior to implanting them subcutaneously into host mice of similar age and strain in a manner which has been described elswhere (Horning, 1947). Usually 4 subcutaneous grafts were made on each side of the abdomen of a single host mouse in order to have the implants growing under identical conditions. Small palpable tumours were obtained at intervals ranging from 6–12 weeks following grafting, but in order to examine the differences between the responses to individual carcinogens, grafts were fixed before tumours developed at intervals ranging from 1–5 weeks after implantation.

Lung fragments from 6½ weeks old Strain A mice were grafted, in some instances without the carcinogen, into host mice of similar age, and also into mice of 15 months age which were selected from stock mice of undetermined ancestry. This was done to find out if the survival of homologous grafts was dependent on employing a closely inbred strain, or whether it might be influenced by the age of the host.

In another series of experiments a number of lung grafts were treated with 20-methylcholanthrene, half being implanted into the superficial fascia, and the remainder placed as usual between the skin and the abdominal wall. The host mice were again of the same age and strain, and it was thought that some information might be obtained as to whether rapid vascularization played any role in successful survival of the grafts.

TABLE I.

Donor mouse.			Number of mice.	Host mouse.			Carcinogen.	Grafts.			Number of tumours.	Histology of grafts.
Strain.	Sex.	Age (weeks).		Strain.	Sex.	Age (weeks).		Number of grafts.	Period of implantation (weeks).	Number of survivals.		
A	♂	6½	30	A	♂	6½	None	60	8	36	None	36 grafts owing to failure of vascularization in host tissues became necrotic; remainder were totally absorbed.
A	♀	6	30	A	♀	6	„	60	8	40	„	18 grafts became absorbed in tissues of host mice. Two others were necrotic.
A	♀	32	30	A	♂	32	„	59	8	14	„	14 grafts survived; 4 contained areas of necrosis.
A	♂	6½	30	Hybrid	♂	40	„	60	8	2	„	58 grafts became totally absorbed in tissues of host mice.
A	♂	6½	15	A	♂	8	Commercial cholesterol	15	9	8	„	Foreign body reaction. Many grafts contained multinucleated giant cells.
A	♂♀	7½	16	A	♂♀	7½	20-methyl-cholanthrene	31	8–11	18	18	Bronchogenic carcinoma. Remaining 13 grafts either became absorbed or were necrotic.
A	♂	12	20	A	♂	11	20-methyl-cholanthrene	20	12 implanted into superficial fascia	None	None	All grafts failed to become vascularized and were in process of absorption.
A	♂	8	15	A	♂	10	2-acetyl amino-fluorene	30	8–12	16	12	12 adenomas. Remaining grafts were either necrotic or absorbed.
A	♂	8½	16	A	♂	9	2-amino-fluorene	25	8–13	20	13	11 adenomas, 2 early bronchogenic carcinomas.
A	♂♀	7½	15	A	♂♀	8	20-methyl-cholanthrene	30	1–5	28	None	2 grafts necrotic; remainder showed bronchiolar proliferation.
A	♂♀	8	16	A	♂♀	7½	2-acetyl-amino-fluorene	25	1–5	22	„	3 grafts were absorbed.
A	♂♀	7½	15	A	♂♀	8	2-amino-fluorene	25	1–5	21	„	2 grafts had become necrotic, 1 had been absorbed.

Survival of lung grafts without the carcinogen.

Lung tissue was obtained from 30 Strong A male mice aged 6½ weeks, and implanted subcutaneously into host mice of the same strain and age, each individual receiving 2 grafts on each side of its belly (Table I). All the grafts were recovered after 8 weeks' implantation and fixed for histological examination. Out of a total of 60 grafts 36 had survived and the remainder had failed to become vascularized (Table I). Female mice of the same strain received the same number of lung grafts from female donors in order to ascertain if sex influenced the result. These grafts were also fixed after 8 weeks' implantation. In this case 40 grafts survived, and of the remainder 18 were absorbed and 2 had become necrotic. When the same number of grafts from females were implanted into Strong A male mice over 8 months of age, only 14 grafts had survived after 8 weeks and 4 of these contained areas of necrosis (Table I). Grafting the same number of lung implants from Strain A mice 6½ weeks old into stock mice of indeterminate ancestry, all of which were over 10 months old, resulted in 58 becoming totally absorbed after the same interval of 8 weeks' implantation (Table I). Necrotic grafts are easy to recognize macroscopically since they are no longer translucent like healthy grafts, being opaque white or yellowish in appearance. The cellular changes in the necrotic cells of the bronchiolar epithelium appear to be of two kinds. Either the nuclei become pycnotic, or as in most grafts they lose their differential staining and the cytoplasm becomes homogenous, to be followed in later stages by fragmentation and dispersal. The nuclei of the collapsed lung alveoli undergo chromatolysis far more rapidly than those of the adjacent bronchiole epithelium. Histological examination of these grafts suggests that their failure to survive in host tissues may be due to a partial failure of vascularization which induces necrosis. These results obtained with adult lung grafts indicate that successful survival and growth depends on age of both the donor and host animals, together with employment of a closely inbred strain in which homologous grafting is much better tolerated than in mixed strains.

Lung grafts combined with cholesterol.

In order to obtain a foreign body reaction fragments of lung from 6–7 weeks old donor Strong A mice were treated with crystals of commercial cholesterol and subcutaneously implanted in 8-weeks-old host mice (Table I).

Grafts combined with the carcinogen.

In every instance in this series of experiments lung tissue was obtained from Strong A mice of either sex, 7–8 weeks old, and impregnated with the carcinogen and implanted subcutaneously into host mice of the same strain and age (Table I).

20-*methylcholanthrene.*—Out of a total of 31 grafts combined with this particular carcinogen, small palpable primary growths arose after 8–11 weeks at the site of implantation. Eighteen tumours were removed at the end of the 12th week. The remaining 13 were no longer translucent, and it was found that eight of these opaque grafts had been accidentally transplanted into the superficial fascia instead of between the skin and the abdominal wall. On histological examination it was clear that vascularization had failed. An additional experiment was undertaken in which a further 20 lung grafts were combined with the carcinogen and

purposely transplanted into the superficial fascia. When no palpable lesions appeared after the 12th week all grafts were removed for histological examination. Sections were cut through each graft with the skin *in situ* to permit examination of the lung implant in relation to the host tissues. These findings confirmed the contention that the failure of the grafts to survive was due to their inability to become vascularized (Fig. 2, Table I).

2-*Acetylaminofluorene*.—Out of 30 grafts made with this carcinogen two small hard nodules appeared at the end of the 8th week following implantation, and were removed for histological examination. After 12 weeks a further 10 small lesions appeared, and these were fixed together with the remaining lung grafts which had failed to produce tumours (Fig. 9, 10, Table I).

2-*Aminofluorene*.—Twenty-five lung grafts were implanted with this carcinogen, and from these three small nodules appeared during the 8th week, while 2 other lesions in the same host had ulcerated through the skin during the 9th week. At the end of the 11th week an additional 6 small tumours arose and were removed. The remaining 12 implants were found at autopsy to have degenerated (Table I).

Histology of Pulmonary Tumours.

The histological characteristics of pulmonary tumours induced from subcutaneous lung grafts treated with carcinogens differ in some respects from those of tumours arising *in situ* in the intact lung of rodents under the influence of similar inciting agents. In pieces of isolated lung growing as homologous implants in host animals the alveoli are collapsed, the concentration of the carcinogen is much higher, and the organ is no longer exposed to aerial contamination. Tumours develop in a much shorter time than they do in the intact lungs of animals treated with carcinogens at some remote site.

A typical carcinoma of the lung in a graft impregnated with 20-methylcholanthrene fixed 11 weeks following subcutaneous implantation in a Strong A host mouse is illustrated in Fig. 1 and 3 (Table I). Histological examination shows conclusively that these tumours were derived from the bronchiolar epithelium and were therefore bronchogenic carcinomas. The malignant cells are found to retain their cilia, which is additional evidence of their origin (Fig. 3, 4). The small pieces of muscle in the periphery of the implant (Fig. 1M) are portions of the abdominal wall of the host; cartilage (Fig. 1C) shows that this particular fragment of lung contained portion of a bronchus. Irrespective of whether the fragments from donor mice were selected from the region of the bronchi, or from more peripheral areas in the lung, and irrespective of the particular kind of carcinogen employed, the tumours were found always to arise from the bronchiolar epithelium (Fig. 1, 3). In many primary grafts, regardless of the type of carcinogen used, compact neoplastic foci comprising small acini lined with ciliated malignant cells are seen lying adjacent to collapsed lung alveoli (Fig. 5, 7), suggesting that the carcinogen possesses a selective action only for the cells of the bronchiolar epithelium, since the cells of the alveoli appear to remain entirely quiescent and unaffected by the local presence of the carcinogen. This observation will be the subject of further discussion later.

In some early pulmonary grafts, malignancy commences in the epithelium forming the bronchial wall. In the graft illustrated in Fig. 11 and 12 the early malignant cells are derived directly and exclusively from the epithelium. This

particular graft had been treated with 2AAF, and was recovered from the host 7½ weeks following implantation. The lumen of the bronchiole is occluded by cellular debris, and the epithelium in some regions has become stratified, but in the malignant area the bronchiolar epithelium is ciliated. The cells comprising this neoplastic focus possess all the histological characteristics of malignant cells. Their boundaries are indistinct, the cytoplasm is highly granular, multinucleated cells are present, and abnormal mitoses are common (Fig. 11).

The histological appearance of these carcinomas varies, however, according to the age of the primary graft. Those recovered from mice 9–12 weeks old consist of acini of cells with large oval nuclei. In older malignant grafts of over 14 weeks after implantation the carcinomas are composed of more solid acini with spherical nuclei in the cells which lose their cilia. These tumours often begin at this period of growth to infiltrate beyond the boundary of the implant and invade the connective tissues of the host. Lymphatic infiltration sometimes seen in earlier grafts of 8–12 weeks age is entirely absent in these older implants.

All primary tumours induced by the several carcinogens were obviously bronchogenic carcinomas, with the exception of a squamous-celled growth induced by 2AAF after 16 weeks' implantation. This tumour was too far advanced to determine its origin, and was composed chiefly of polygonal cells with prickle borders and a tendency to form keratin pearls. Another advanced tumour was an adeno-carcinoma induced by 20-methylcholanthrene and recovered after 17 weeks' growth. This tumour subsequently underwent squamous metaplasia during a third generation of serial transplanting. Apart from these carcinomas there were also two sarcomas, both of which were induced by 20-methylcholanthrene, and fixed after 16 weeks' growth. One was a polymorphic and the other a spindle-celled sarcoma. As both these tumours were too far advanced in their growth it was impossible to ascertain whether they were derived from the tissues of the implant or from the host, so they were discarded. The possibility that they arose from the host, is more likely, since sarcomas of the lung are rare (Willis, 1948). Examination of pulmonary tumours from lung grafts from both sexes growing in mice of either sex showed no difference in the incidence of neoplasia.

Another advantage of this method is that several grafts of lung tissue obtained from a lobe of the same donor can be grown under identical conditions in the same host. In some which carried six or more grafts it was frequently noticed that after 8–10 weeks the palpable grafts varied considerably in size, and that this variation often occurred irrespective of the particular carcinogen employed. Histological examination showed that grafts which had grown the more vigorously were early bronchogenic carcinomas. Those implants which failed to develop into tumours, and were hardly palpable in the living host, were found to contain large inflammatory foci or areas of foreign body reaction. The grafts with inflammatory foci established at relatively early periods of growth had become successfully vascularized, and the collapsed lung alveoli adjacent to the areas of inflammation were composed of healthy cells (Fig. 9, 10). The nuclei of alveolar cells exhibited no chromatolysis or degeneration, the cytoplasm was non-vacuolated and the cell outlines were clearly marked. Likewise the bronchiolar epithelium in these grafts was also composed of healthy cells invariably showing active mucous secretion (Fig. 8). None of the lung grafts with excessive inflammation, irrespective of the type of carcinogen used, was found to develop malignant foci, or the typical proli-

feration of bronchiolar epithelium which constitutes such a striking feature of early healthy grafts following treatment with carcinogens. In every instance the inflamed grafts were obtained from the same donor mouse grown in the same host and treated with the same carcinogen for a similar period of time as those which developed into tumours.

In the majority of grafts (Table I) necrosis and fibrosis are absent, although these changes are so often associated with inflammation of the intact lung *in situ*. Bronchioles when present in these grafts tend to be obscured, since the lumen is filled with cellular exudate consisting mainly of lymphocytes, plasma cells, and a few macrophages or polymorphs. The blood vessels in the inflammatory foci are greatly dilated especially in the grafts combined with cholesterol crystals. This may be due to the direct action of the carcinogen (Hieger, 1949) on the newly formed vessels of the graft (Table I). Multinucleated giant cells frequently seen in tissues reacting to foreign bodies were absent in grafts treated with carcinogen, but were found in several implants impregnated with commercial cholesterol. Many of these grafts were infiltrated with polymorphonuclear leucocytes.

EXPLANATION OF PLATES.

All photomicrographs are of sections of lung grafts treated either with or without a carcinogen. The grafts were transplanted subcutaneously into host mice, and were recovered at intervals ranging from 8 weeks to 12 weeks after implantation. Every lung graft was fixed in alcoholic Bouin and stained by Ehrlich's haematoxylin and eosin.

FIG. 1.—A typical bronchogenic carcinoma, induced from a graft of adult normal lung impregnated with crystals of 20-methylcholanthrene, fixed 11 weeks following subcutaneous implantation in a host (strain A) mouse. C, cartilage; M, muscle.

FIG. 2.—Lung fragment from a donor strain A mouse grafted into the superficial fascia of a host Strong A mouse, fixed 12 weeks after implantation. This graft is in the process of becoming absorbed by the tissues of the host.

FIG. 3.—Carcinoma of lung, induced from a graft of normal adult lung obtained from a donor strain A mouse, impregnated with 20-methylcholanthrene, prior to subcutaneous implantation into a host Strong A mouse.

FIG. 4.—Enlarged area of Fig. 3 (marked by square), showing ciliated malignant carcinoma cells. The lumen shows an occlusion of cellular exudate.

FIG. 5.—Carcinoma induced from lung graft treated with 20-methylcholanthrene, fixed 14 weeks after subcutaneous implantation in host mouse. In area of graft marked by square (Fig. 7) collapsed lung alveoli unaffected by local presence of carcinogen are seen lying adjacent to carcinoma.

FIG. 6.—Lung graft treated with 20-methylcholanthrene which failed to develop into a tumour. Note large inflammatory foci. Bronchiolar epithelium (marked with square) is healthy, and may be seen under a higher magnification in Fig. 8.

FIG. 7.—Primary tumour in lung graft treated with 20-methylcholanthrene (same as Fig. 5); compact neoplastic foci comprising small acini lined with ciliated tumour cells are seen lying adjacent to collapsed lung alveoli. This is of special significance, as it indicates that the carcinogen has a selective action only for the bronchiolar epithelium. The adjoining alveolar cells remain quiescent to the local presence of the carcinogen.

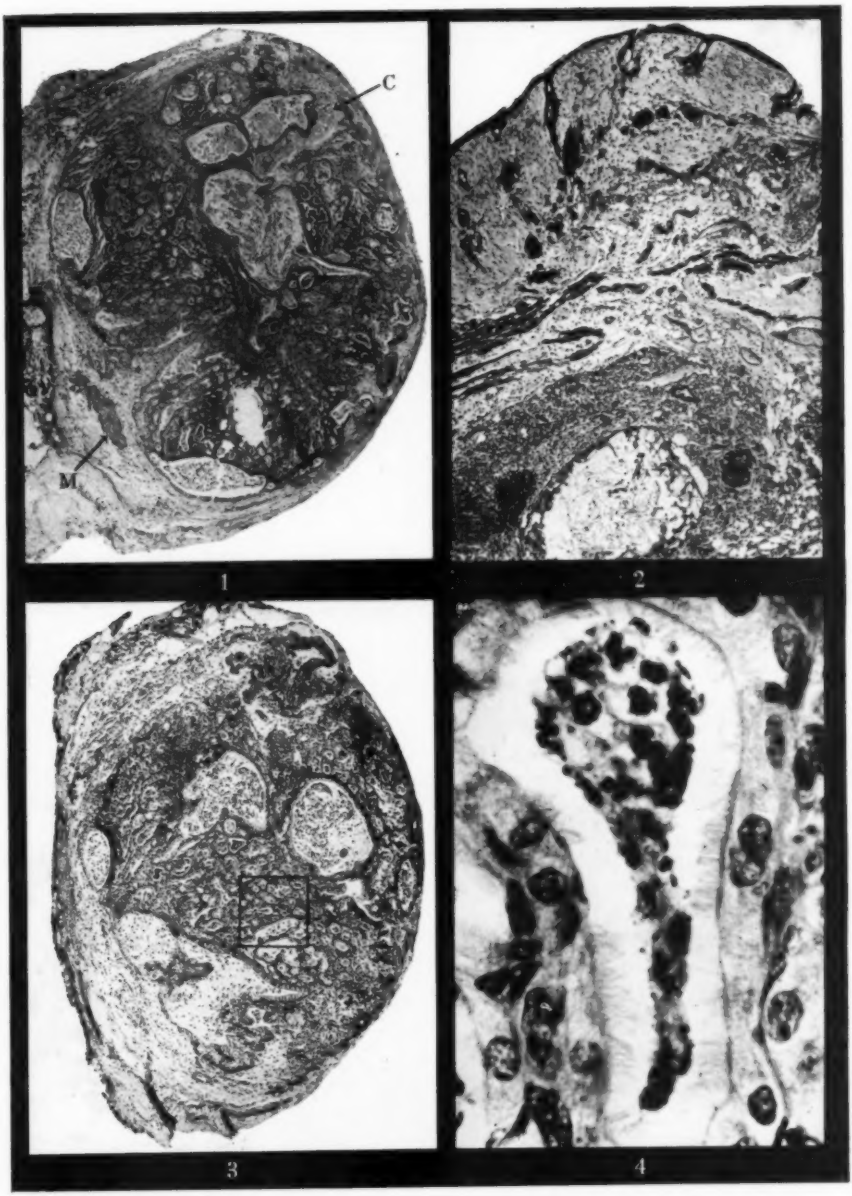
FIG. 8.—Same as seen in Fig. 6, showing cytologically healthy bronchiolar epithelium in graft combined with 20-methylcholanthrene, which failed to develop into a tumour. × 1000.

FIG. 9.—Graft treated with 2-acetylaminofluorene which failed to develop into a tumour. Note inflammatory foci and adjacent areas of healthy collapsed lung alveoli (Fig. 10).

FIG. 10.—Higher magnification of area marked in Fig. 9. The alveolar nuclei are cytologically healthy, exhibiting no evidence of either chromatolysis or cytoplasmic degeneration. Showing polymorphonuclear leucocytes with macrophages and lymphocytes.

FIG. 11.—Lung graft treated with 2-acetylaminofluorene fixed 7½ weeks after subcutaneous growth in host mouse. The malignant focus in this instance originated in the epithelium forming the bronchial wall (Fig. 12).

FIG. 12.—Higher magnification in area marked in Fig. 11. The lumen of the bronchiole is occluded with a cellular exudate, and in some regions the epithelium has became stratified, but in area of malignant focus the bronchiolar epithelium is ciliated. Cells forming neoplastic focus have lost cell boundaries, cytoplasm is highly granular, multinucleated cells are present, together with abnormal mitoses.



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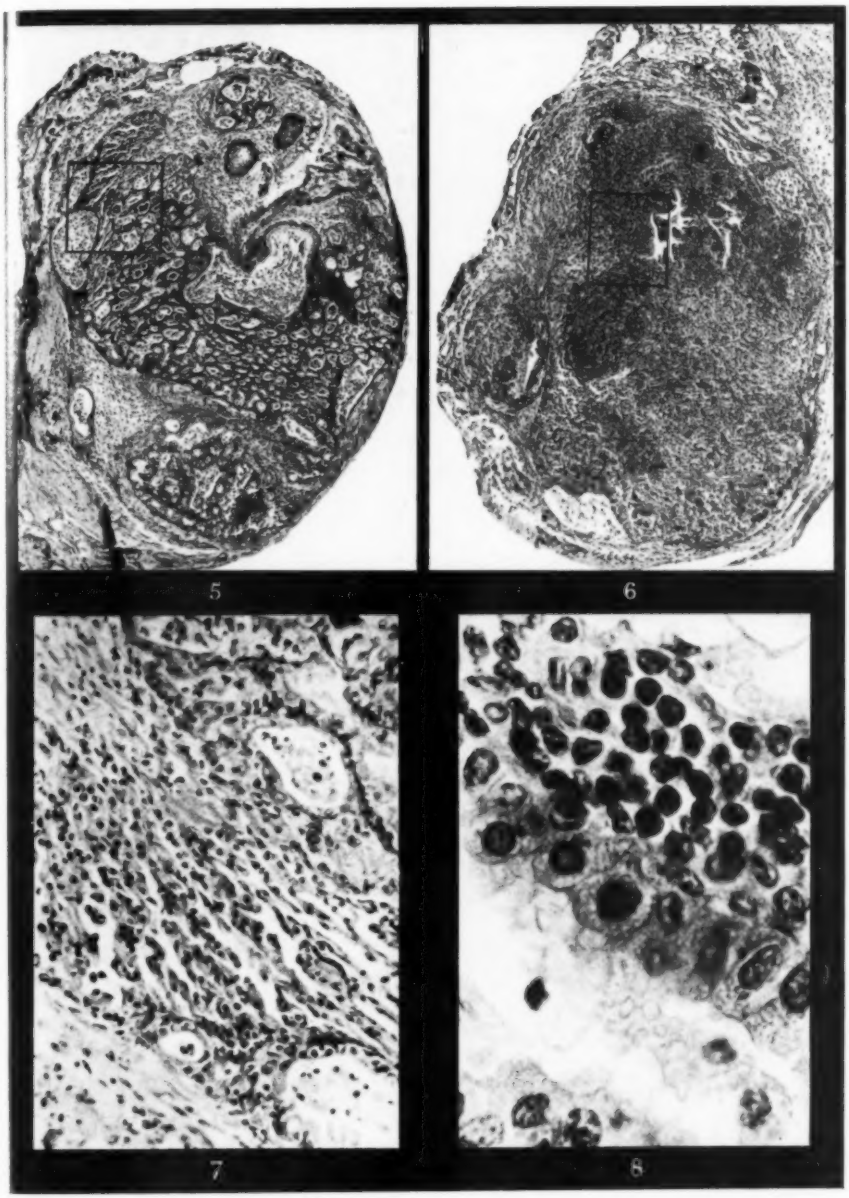

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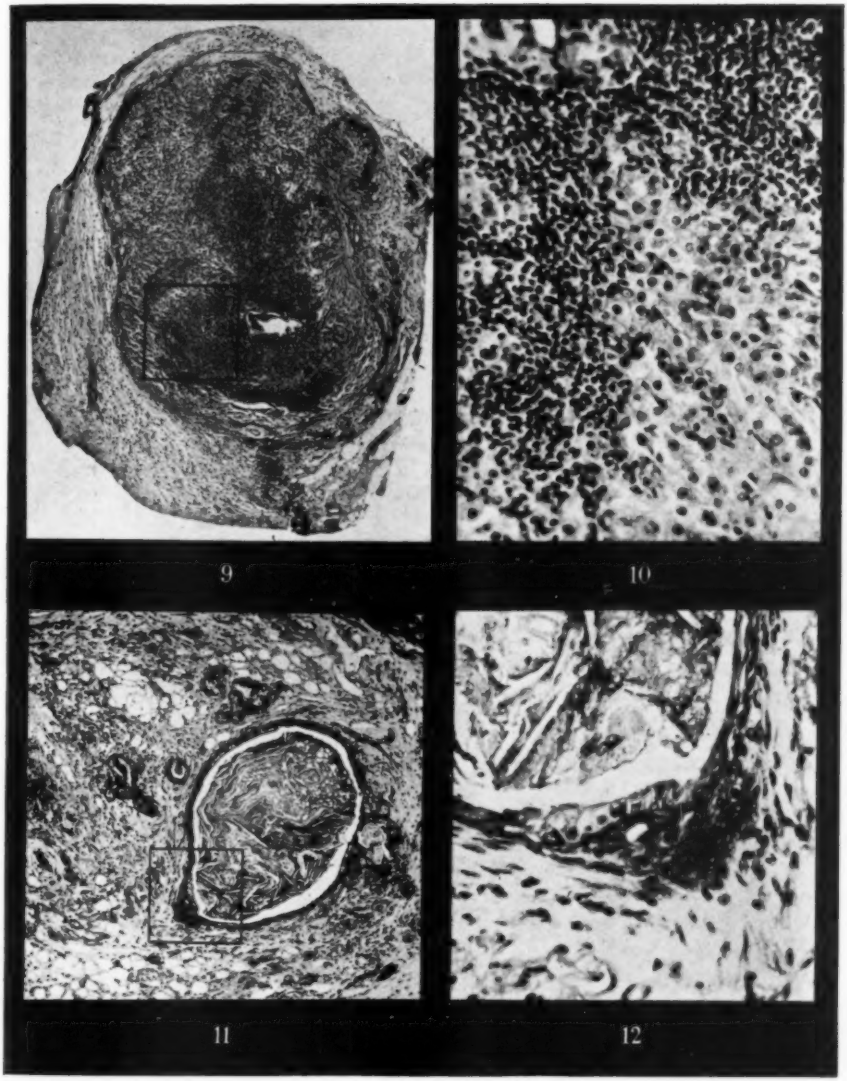

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DISCUSSION.

Since the induction of carcinoma of the prostate in mice, arising from fragments of adult glandular epithelium combined with the carcinogen growing as homologous subcutaneous grafts in host mice, was first reported (Horning, 1946), this method of tumour induction has been successfully extended by other investigators to other tissues and organs. Pan and Gardner (1948) reported the induction of malignant tumours from grafted fragments of mouse uterine cornua and cervices treated with 20-methylcholanthrene. This was of particular interest, as spontaneous malignant tumours of the uterus rarely occur in mice. More recently Hughes (1949 *a*, *b*), using the same technique, has succeeded in inducing transplantable carcinomas from fragments of adult bladder and uterine epithelium combined with methylcholanthrene, as well as tumours of adrenal cortex.

One of the several advantages arising from homologous subcutaneous grafting in the presence of a carcinogen is that the tumours growing under the skin are palpable, and can readily be obtained at the required stage of growth. This is particularly advantageous in the case of the lung. The time taken for tumours to appear is shorter most likely because the carcinogen is more concentrated, and lies in close proximity to the bronchiolar epithelium of the graft. With indirect methods of induction such as painting the carcinogen of the skin, by injecting it or including it in the diet, there may be changes in organs and tissues throughout the body, and not necessarily only in the region where a tumour arises. Effects of this kind may complicate the interpretation of the incidence of a particular kind of tumour. Thus Dunlap and Warren (1942) have reported that if Swiss mice are injected subcutaneously with carcinogenic hydrocarbons, the incidence of primary pulmonary tumours is higher in mice which have also developed tumours at the site of inoculation.

It must be admitted, however, that these two methods of induction differ fundamentally, because the changes which occur in the intact lung *in situ* following treatment at some remote site with a carcinogen are not necessarily the same as those which take place in homologous subcutaneous grafts of isolated, collapsed lung. These differences were discussed in a recent paper by Orr (1947), in which he rightly pointed out that it is difficult to compare results obtained by the two methods of induction.

Recent experiments have shown conclusively that the survival of lung grafts either with or without the carcinogen is dependent upon the age and strain of the donor mouse, together with the age and strain of the host. Examination of Table I shows, however, that donor lung implants impregnated with a carcinogen survive more readily and in greater numbers in older hosts than would be the case if the grafts did not contain any carcinogen. These results confirm previous experience with grafts of prostatic epithelium under the same conditions (Horning, 1949). Similarly Pan amd Gardner (1948) found an increased susceptibility to tumour formation from grafted uterine epithelium in Strain A mice as compared with hybrids. For reasons which are still obscure more difficulty has been experienced in obtaining malignant tumours from lung grafts impregnated with 20-methylcholanthrene than with similar grafts of prostatic tissue. It is of course possible that since the lung carcinomas arise from bronchiolar epithelium, some grafts may at the outset contain insufficient epithelium of this region to give a response. Perhaps also the location of the carcinogen within the grafts may be

unsuitable for full effect on the bronchioles. The bronchioles may be damaged in isolating the fragments, or there may be partial failure of vascularization. A separate series of experiments in progress show that pulmonary tumours are obtained more readily and in greater numbers from fragments of lung in which particular care has been taken to isolate portions of a bronchus, discarding the more peripheral parts of the organ. It seems likely that direct contact between the bronchiolar epithelium and the carcinogen is an important factor in tumour induction. Otherwise it is difficult to understand why prostatic epithelium should flourish as a graft despite the antagonism between graft and host tissues, and yet lung fragments implanted under the same conditions do not always survive so readily. Preliminary experiments reported elswhere (Horning, 1949) suggest that tissues which are normally under hormonal influence are much better tolerated as grafts in host mice of the same sex and strain than tissues which are to a greater extent independent of hormone action.

It will be noted that lung tumours induced in grafts arose with equal facility in males and females. A higher incidence was not obtained in males, as seems to be the case in human lung cancer according to Clemmensen and Busk (1947), and in Strain A and Strain C mice treated with urethane in the experiments of Larson and Heston (1945). As many as 50 per cent more lung tumours in males were reported in mice by these investigators. There is a slight difference between the potency of the three carcinogens used in the present experiments in that a greater number of tumours appeared in grafts treated with 20-methylcholanthrene. The interval between implantation and tumour induction is shorter in the case of 2AAF.

With regard to the histogenesis of the present lung tumours the method of grafting allows examination of the neoplastic changes in graded stages, leaving no doubt that the tumours arise from bronchiolar epithelium. It is curious that no response to the carcinogens is obtained from the walls of the lung alveoli. If an alveolar epithelium exists why should it remain entirely quiescent, whilst the closely adjacent epithelium of the bronchioles proliferates in so striking and so specific a manner ? Controversy still exists concerning the nature of the lining of the lung alveoli. Ross (1939) maintains that although an epithelial lining to the alveoli is not visible in ordinary histological preparations, it becomes apparent in response to certain abnormal stimuli. Grady and Stewart (1940) not only affirmed the existence of an alveolar epithelium, but regarded it as the source of lung tumours in mice treated with methylcholanthrene. According to Wells, Slye and Holmes (1941), lung tumours in mice differ from those which occur in man. Thus while they agreed that human tumours are bronchogenic in origin, they were convinced that mouse lung tumours arose from the alveolar epithelium. More recently Herbut (1946) has reviewed the evidence concerning the origin of human lung tumours, and concludes that all " alveolar cell tumours " come from the basal cells of the bronchi and bronchioles, and that there is no justification for assuming that they arise from septal cells. It is unlikely that any fundamental difference exists between the rodent and human lung, and since there is such clear indication of the origin of malignant foci in early grafts combined with a carcinogen, it would appear that the case for bronchogenic origin of all lung carcinomas is strengthened. Support for this contention is also given by Orr and Bielschowsky (1947) in their studies on the histogenesis of rodent lung tumours. Gunn (1948) further points out that tissue culture studies and numerous experiments *in vivo*

demonstrate that the nucleated cells found on the surface of the alveoli are indistinguishable from macrophages, and the non-nucleated "epithelial plates" are probably artefacts arising from silver impregnation techniques which, according to Loosli (1937, 1938), are confused images of the outlines of the endothelial cells of the capillaries. In the presence of chronic inflammation a proliferation of the epithelium in the respiratory bronchioles may extend to form a lining within the alveolar ducts and alveoli in much the same manner as bronchial epithelium will grow into an old abscess cavity. These observations all support the view of those, such as Barnard and Day (1937), who contend that there is no lining to the pulmonary alveoli.

The relationship of pulmonary cancer to inflammatory foci has been studied recently by Grady and Stewart (1940), De Eds and Cox (1941), Nettleship, Henshaw and Meyer (1943), Orr (1947), Orr and Bielschowsky (1947), and Selbie and Thackray (1948). The effects of urethane on the mouse lung *in situ* indicate according to Nettleship, Henshaw and Meyer (1943) and Orr (1947) that inflammation and adenomas arise together. Grady and Stewart (1940) and Selbie and Thackray (1948) disagree, and the last authors believe that in Orr's series of 433 animals which were examined after they had died from natural causes, there was a susceptibility to terminal inflammatory involvement. Orr (1947) also found, however, that there was a similar association in lung tumours in mice following induction by chemical agents. Here also the evidence obtained from grafting clearly supports the contention that there is no relationship between inflammation and lung neoplasia, since the grafts which failed to produce tumours contained large areas of inflammation and those in which tumours developed rapidly were comparatively free. It is difficult to say why some grafts developed large inflammatory areas while others growing in the same host were unaffected. Perhaps the variable amount of injury entailed in dissection and transplantation may account for this phenomenon.

SUMMARY.

Bronchogenic carcinomas have been induced from homologous subcutaneous grafts of lung tissues growing in host mice, after impregnation with either 20-methylcholanthrene, 2-acetylaminofluorene, or 2-aminofluorene.

The period of tumour induction in grafts of homologous lung is considerably reduced when compared with lung tumours which develop *in situ*, in rodents following treatment with the carcinogen at some remote site on the body. The factors responsible for this acceleration of carcinogenesis in lung grafts have been discussed.

The susceptibility to tumour formation in subcutaneous lung grafts treated with the carcinogen is found to be dependent upon the age and strain of the donor as well as that of the host mouse. Lung tumours develop more frequently in inbred than in hybrid mice, irrespective of the type of carcinogen used.

The histogenesis of lung tumours has been described, and the relationship between inflammatory foci in grafts and the subsequent development of lung cancer has been investigated. Additional experimental evidence supports the view that the pulmonary alveoli are not lined by a continuous epithelium.

Factors influencing the survival of lung grafts without using a carcinogen were also investigated ; these showed that the successful survival of subcutaneous grafts is dependent upon the age of both the donor and host mice, together

with the employment of a closely inbred strain of mice, in which homologous grafting is much better tolerated than in mice of mixed strains.

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STRAIN DIFFERENCES IN MICE TO THE CARCINOGENIC ACTION OF URETHANE AND ITS NON-CARCINOGENICITY IN CHICKS AND GUINEA-PIGS.

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In a previous paper (Cowen, 1947) note was made of the difference in response by various lines of mice to the carcinogenic action of urethane. RIII mice developed more lung tumours than CBA mice and both developed more than C57 mice. This is the same order as the incidence of spontaneous lung tumours in these strains of mice. A further and more detailed study of this strain difference was undertaken. For this purpose 2 strains, A and C57 black, were chosen, which show a large divergence in respect of spontaneous lung tumours. Strain A mice have a spontaneous lung tumour incidence of about 89 per cent, and C57 mice less than 1 per cent (Bittner, 1938).

Although urethane has been mentioned as carcinogenic for mouse (Nettleship and Henshaw, 1943) and rat lung (Jaffé, 1947) this action has not yet been reported in other animals. Chickens and guinea-pigs were therefore given prolonged treatment with urethane in order to determine if they were susceptible to its carcinogenic action.

MATERIAL AND METHODS

In order to study the inheritance of the strain difference, A and C57 mice were crossed to give their F1 and backcrosses. Since the inherited susceptibility to most known cancers is due to dominant genes this was anticipated here, and consequently a large number of the C57 backcross mice were bred in order to note segregation, which should be manifest in the group on this assumption.

The urethane was administered by dissolving it in the drinking water of the mice. This proved to be effective in eliciting tumours by Henshaw and Meyer (1945) and Cowen (1949). Preliminary experiments indicated that the various strains and crosses of mice used did not differ in their fluid intake, and consequently strain differences due to this treatment were not due to a different intake of the carcinogen.

All the mice were approximately 2 months old at the start of the experiment. Their food consisted only of the 14 per cent rat cake nuts as supplied by the North-Eastern Agricultural Co-operative Society, Aberdeen. The mice were given a 0·1 per cent solution of urethane in tap-water in place of their drinking water. No other water was available since the food of the mice was dry. After 3 months of this urethane treatment the solution was replaced by tap-water for 4 months, after which time the mice were sacrificed. The urethane given to all the mice came from the same stock solution, which was made up in 10 litre quantities at a time.

The numbers of mice used, their strain, and the numbers surviving treatment are given in Table I.

TABLE I.—*Strains, Numbers and Survival Rates of Mice Treated with Urethane.*

Strain of mouse.	Numbers at start of experiment.	Numbers surviving experiment.	Survival (%).
A	44	15	34
C57	25	22	88
A × C57	28	23	82
A × (A × C57)	33	15	45
C57 × (A × C57)	98	82	84

Eight of the 23 surviving A × C57 mice did not receive exactly the same treatment as the others. They were from another experiment and were only given 7 weeks' urethane treatment, though they were killed 7 months from the beginning of treatment, the same as the rest. They are included in the data, since they gave the same lung tumour response as the F1 mice which received full treatment. It is suggested that the lower dose was one causing maximum effect.

When the mice were sacrificed their lungs were removed and fixed in 10 per cent formol-saline for 24 hours, which preserved the material and made handling easy. Since nearly all lung tumours in mice tend to occur on the periphery of the lobes (Grady and Stewart, 1940) the lobes were separated and the number of nodules on their surface visible to the naked eye were counted. This was taken to be the index of the response to urethane. When the number of tumours in a lung exceeded 80 there was liable to be some error in this figure. Such error, however, does not affect the results on the whole, since only 7 mice altogether had lung tumour counts higher than this number.

As a check it was decided to use another criterion of response. In a previous paper (Cowen, 1947) it was noted that strains of mice which gave a low number of lung tumours had tumours of a smaller size. The size of the lung tumours were therefore also studied to give an index of response to the carcinogenic action of urethane. Since the tumours in a lobe varied in size from microscopic dimensions to nodules about 4 mm. in diameter, it was not feasible to use the average size of all these tumours. The best way of gauging the response on a basis of tumour size was considered to be that of determining the size attained by the largest tumour in the total lungs of a mouse. The material already obtained for lung tumour counts had been preserved in formalin with the lobes separated. Each lobe was examined under a low power dissecting microscope which had an eyepiece micrometer. The diameter of the largest tumour on the surface of each lobe was measured. Most of the tumours were circular in plan. Others were elliptical, and their size was taken to be the average of the major and minor axes. The diameter or average diameters were taken to be the measure of size of the tumours.

Preliminary experiments showed that lung tumours shrank slightly during their first 24 hours in the fixative but not after. The amount of shrinkage was proportional to the size of the tumour. No significant error was thus considered due to the formalin, since all tumours were measured after at least 24 hours' fixation.

RESULTS.

Mice treated with urethane.

The tumours consisted of raised circular nodules with a discrete edge and had a "pearly white" appearance. One A, one C57 and one C57 backcross mouse surviving the experiment had lungs which presented a hepatized appearance. Although tumours were present, an accurate count could not be made because of this condition and no figures were obtained for these mice.

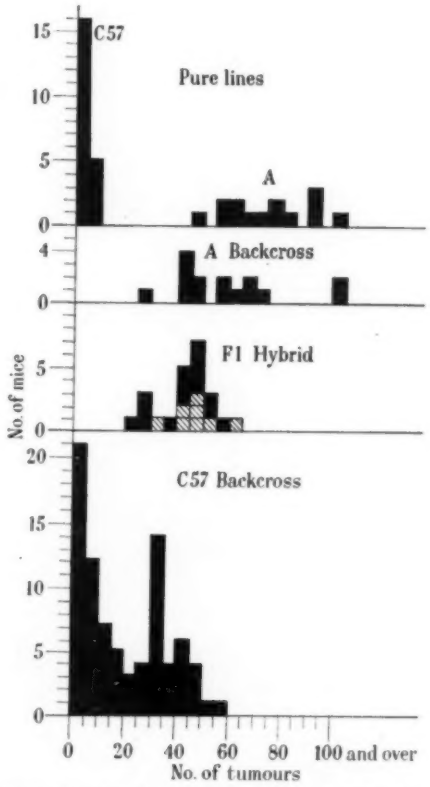

FIG. 1.—Frequency polygon showing the response of A and C57 mice and their crosses to urethane on a basis of number of tumours. The shaded squares represent mice which received slightly different treatment as explained in the text.

The results of the lung tumour counts and sizes are shown in Fig. 1 and 2 respectively. The shaded squares representing F1 mice indicate those which had less treatment. As can be seen, they responded in the same way as those mice which received full treatment.

As Fig. 1 indicates, there is a very distinct difference between the two pure lines in response to identical treatment with urethane. The response of the A

and A backcross mice are similar. This was tested statistically using the formula $\frac{m_1 - m_2}{\sqrt{E_1^2 + E_2^2}}$. On the other hand, the F1 hybrids were slightly but significantly lower than both of these classes. The polygon for the C57 backcross mice is obviously bimodal, and roughly consists of a combination of the figures for the C57 and F1 hybrid mice in approximately equal proportions.

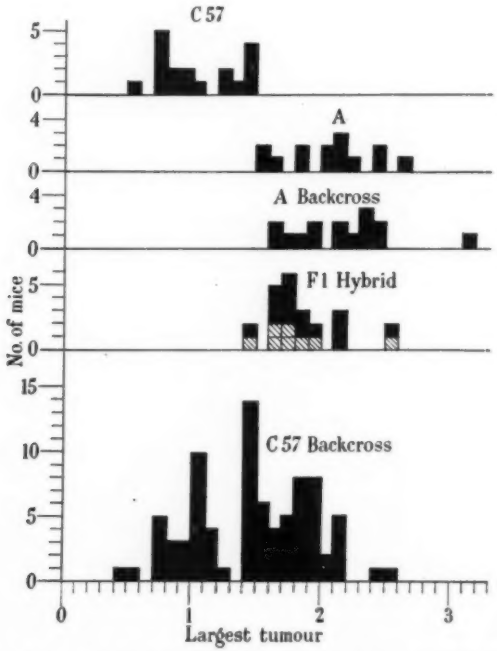

FIG. 2.—Response of A and C57 mice and their crosses to urethane on a basis of the size of the largest tumour per mouse. The shaded squares represent mice which received slightly different treatment as explained in the text.

The same results are obtained using sizes as the criterion of response (Fig. 2), though the differences are not so marked as in Fig. 1. Furthermore there is less tendency for the polygons to form normal distribution curves than in Fig. 1. Treating the figures statistically as before, the pure lines showed a significant difference. The A and A backcross mice again showed the same response, but the F1 hybrids tended to have smaller tumours. The C57 backcross mice again give a bimodal polygon.

At first no correlation was made between the numbers of tumours in individual mice and the sizes of the largest tumours. When it became apparent that the C57 backcross were segregating into 2 groups, such note was kept for the 44 remaining mice. The number of tumours in a mouse of this cross was plotted against the size of the largest tumour in the same mouse to give the scatter

diagram in Fig. 3. It can be seen that those C57 backcross mice which had many tumours had larger ones and vice versa.

It may be mentioned that the A backcross mice which segregated for colour giving three phenotypes, albino, non-agouti and non-agouti brown, showed no apparent linkage of susceptibility with colour.

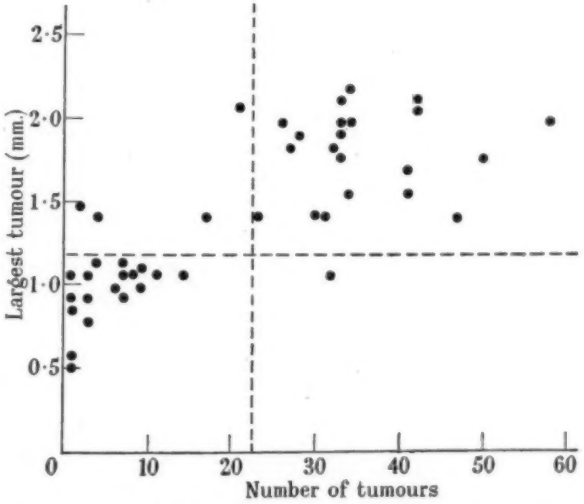

FIG. 3.—Scatter diagram showing the relationship between the 2 groups of C57 backcross mice with respect to number and size of tumours. The dotted lines represent the division into the 2 segregating groups.

Chickens and guinea-pigs treated with urethane.

The chickens used were brown leghorns, from the flock maintained by Dr. Greenwood at the Poultry Research Centre, Edinburgh. Six young adult hens were injected intraperitoneally with a 25 per cent aqueous solution of urethane as follows: Three c.c. were given thrice weekly. This dose debilitated the birds to some extent because of the prolonged narcosis due to urethane. Two months later 2 of the birds died as a result of this and 2 c.c. instead of 3 were given to the survivors thrice weekly as before. After 4 months of this treatment no more urethane was given for 2 months, during which time one of the birds collapsed. It presented an anaemic appearance and was gasping for breath. It was sacrificed, and on post-mortem examination showed growths in both lungs and the heart. Sections of the growths were made, and diagnosis was kindly undertaken by Dr. J. G. Campbell at Edinburgh. These neoplasms were found to be of lymphoid origin.

When injections were started again (after the rest period of 2 months) 3 c.c. of the 25 per cent solution was given twice weekly. Three birds were left at this time, and they all died within the next 5 months from non-specific causes. No evidence of tumours was found in any of them.

Seven young adult male chickens were given a 0·2 per cent solution of urethane

in tap-water in place of their drinking water. The birds drank this *ad lib.* with no obvious untoward effects till they died or were killed. Two of the birds died at 4 and 6 months after beginning the treatment as a result of having been severely pecked by their mates. On post-mortem examination they were found to be quite healthy otherwise. A third bird died 13 months after the beginning of treatment. Death was due to a pyogenic infection of the wattles in connexion with an ulcerated mouth. The bird had not been feeding. Apart from this the bird was found to be healthy on post-mortem examination. The 4 remaining birds were killed 18 months after the beginning of treatment, and were found to be quite normal and in good condition of health.

Seven young adult guinea-pigs were given a 0·1 per cent solution of urethane, which they consumed at the rate of 50 to 80 c.c. per day. Four of the animals died at $2\frac{1}{2}$, 8, 11, and 12 months from the beginning of the experiment. In at least 2 of these cases death was due to infections. No definite cause of death could be ascribed to the other two. In all cases no evidence of new growths could be found in the lung or elsewhere. Of the 3 remaining guinea-pigs, 1 was sacrificed at 17 months from the beginning of treatment and the other 2 at 19 months. On post-mortem examination the animals appeared to be quite healthy and normal, special attention having been paid to the lungs.

Thus, of all the chickens and guinea-pigs treated with urethane, only one chicken developed a neoplasm which was lymphoid in origin. This type of tumour is not uncommon in chickens normally, and is not considered to be due to treatment with urethane.

DISCUSSION.

Two criteria of tumour response to urethane were used. The first (number of tumours) gave more obvious results than the second (size of the largest tumour per mouse), though both results closely paralleled each other. The second method therefore substantiates the validity of the first. The same conclusions can be drawn from the results obtained from both criteria.

There is a definite strain difference in response to the carcinogenic effects of urethane between A and C57 mice. The A mice had a large number of lung tumours and the C57 mice had few. The F1 mice had a large number of tumours but not quite so many as their A parent. The A backcross mice had as many tumours as the A mice. The C57 backcross mice segregated into 2 groups, with almost equal numbers of mice in each. The low group gave a similar response to the C57 mice but had a larger deviation from the mean, and the high group had a slightly lower lung tumour incidence than the F1 hybrids (Fig. 1).

These results point most significantly to the existence of a single dominant gene in A mice which is responsible for urethane-induced lung tumour susceptibility, the C57 mice carrying the recessive. This does not entirely explain the results, for if a single dominant gene only were responsible, then A, F1, A backcross and the high group of the C57 backcross mice would all have exactly the same tumour incidence, which is not the case.

This deviation from the ideal case is presumably due to other factors. That lung tumour incidence is not an all or none effect in the mice studied shows that incomplete penetrance or expressivity of the gene probably complicates the matter. The presence of modifying genesis also inferred, but their exact nature cannot be determined without repeating these experiments on a larger number

of animals. The results suggest that the greater part of the strain difference studied in the mice is due to a single dominant gene. There are lesser effects exerted by other factors.

All the groups of mice showed some mortality during the experiment, and tumour counts for these mice were not obtained. This may have biased the data, since possibly all mice which died had a greater tumour response than the survivors. Two independent arguments show that such bias would not affect the conclusions. Firstly, the tumour susceptibility was genetically independent of mortality, and secondly, as the mortality was considerable only in the A and A backcross mice, a higher tumour susceptibility in the dead mice would merely have emphasized the line differences.

Heston (1940, 1942*a*, 1942*b*) reported somewhat similar results using spontaneous and hydrocarbon-induced lung tumours in A and L mice (high and low strains respectively). He did not note any segregation in the L backcross mice however. A possible reason for this is that the incidence of lung tumours in these mice was much lower than in the urethane-treated mice of the C57 backcross as reported here. This would tend to mask any signs of segregation. Furthermore, Heston's low strain (L) possibly have a genotype of complicating factors which the C57 mice do not carry.

Lung tumours are very common spontaneous neoplasms in mice, and that there is an inherited susceptibility to them has been known for a long time. Tyzzer (1907, 1909) noted a familial tendency to lung tumours in mice long before inbred mouse strains had been developed for cancer research. Lynch (1926) reported a dominant gene responsible for susceptibility to spontaneous lung tumours. Similar conclusions were reached for hydrocarbon-induced lung tumours (Lynch, 1927 ; Andervont, 1937, 1938 ; Heston, 1940, 1942*b*).

From the results of these workers, it appears that certain carcinogenic chemicals cause different lung tumour incidences in different strains of mice depending on the incidence of spontaneous lung tumours in these strains. That is, a strain of mice which develops few spontaneous lung tumours does not respond so well to a carcinogen as a strain which has a high spontaneous incidence. This applies also to urethane-induced lung tumours as shown here and previously (Cowen, 1947). From this, it seems that environmental factors (certain carcinogens) do not simply cause tumours, but increase the incidence of them in mice depending on the inherited tendency of mice to develop them. This argument can most probably be applied to other animals as well as mice. It was shown here that chickens and guinea-pigs do not give lung tumours on treatment with urethane. It seems rather significant that no reports have been found in the literature of spontaneous (or induced) lung tumours in guinea-pigs, and out of the many neoplasms that arise in chickens, only one report exists of a spontaneous lung tumour in these animals (Apperly,1935). This then leads to the conclusion that in the case of lung tumours, carcinogens hasten a process which tends to occur spontaneously and is genetically determined. Evidence exists in the literature that this may apply to tumours of other organs. For example, guinea-pigs, which have a relatively frequent incidence of spontaneous liposarcomas (Warren and Gates, 1941), develop these tumours on treatment with hydrocarbons (Shimkin and Mider, 1941).

Not only is urethane the most potent known lung tumour inciter in mice, but it acts as a carcinogen on lung tissue only. Assuming it acts by increasing

a normal tendency, it should afford information concerning the processes which are responsible for spontaneous lung neoplasia. An elusive carcinogenic mechanism would be accentuated by urethane, though the fundamental process remained the same. It is also useful that urethane does not complicate studies by inducing tumours in organs other than lung.

In a previous paper (Cowen, 1947) it was noted that mice treated with urethane suffered a heavy mortality due to general infections. Further work (Cowen, 1949) showed that urethane possibly acted as a carcinogen by its leucopaenic properties. It was considered that the resistance of mice to infections in general was lowered, and at the same time a specific carcinogenic virus was responsible for the lung tumours. If urethane acted by this mechanism, then the A mice would be expected to suffer heavy mortality and C57 mice would survive treatment well. This is the case, as seen from Table I. Table I also shows that the F1 and *C57 backcross* mice had a survival rate almost the same as the C57 mice. This obviously points out that resistance to infection does not necessarily accompany resistance to tumours. If a single recessive gene is assumed to be the cause of the different mortalities, then the A backcross would be the segregating group, and should have a survival rate equal to the average of those for the A and C57 mice. This would be a percentage survival of 61. From Table I it is seen to be 45, which agrees reasonably with the theoretical figure considering the small number of mice involved ($\chi^2 = 3{\cdot}17$, $P > 0{\cdot}05$).

It can thus be safely concluded that the mechanism whereby strain A mice are genetically susceptible to lung tumours is not the same as the one responsible for their susceptibility to general infections. Correlation between leucopaenicity causing susceptibility to infection on one hand and a carcinogenic virus causing lung tumours on the other is therefore considered most unlikely in mice given urethane treatment.

SUMMARY.

Two criteria which parallel each other closely are used as an index of response of mice to the carcinogenic action of urethane—the number of tumours per mouse and the size attained by the largest tumour in total lungs.

Strain A and C57 mice show a strain difference with respect to urethane-induced lung tumours which is mainly due to a single dominant gene or gene complex.

Chickens and guinea-pigs do not develop tumours of the lung or other organs on prolonged urethane treatment.

It is pointed out that the incidence of urethane-induced lung tumours depends on the spontaneous incidence of these tumours in all animals studied so far.

The genetical data showed that susceptibility to intercurrent infections resulting from urethane was independent of susceptibility to lung tumours.

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MECHANISM OF OXIDATION OF 3:4-BENZPYRENE IN THE PRESENCE OF AUTOXIDIZING THIOLS.

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EVIDENCE offered by Calcutt (1949) showed that 3:4-benzpyrene is effective in inducing the oxidation of the –SH groups of a variety of compounds, the benzpyrene itself also undergoing oxidation during this process. On the basis of physical properties and fluorescence spectra it was suggested that the benzpyrene derivatives obtained in this fashion were comparable to those obtained as a result of the metabolism of the hydrocarbon by mice. This suggestion is important in view of Crabtree's (1947) conclusions as to the involvement of –SH groups in the carcinogenic process, and also in relation to Powell and Calcutt's (1949) conclusion that the availability of sulphydryl influences the metabolism of benzpyrene. The present paper reports an attempt at the solution of the problem of the mechanism of the oxidative processes undergone by benzpyrene in the presence of autoxidizing thiols.

Studies by Weigert and Mottram (1946*a*, 1946*b*) showed that the initial step in the metabolism of benzpyrene is conversion to either 8:9-dihydro 8:OR_1 –9.OH benzpyrene (BpX_1) or 8:9-dihydro 8:OR_1 –9OR_2 benzpyrene (BpX_2), R_1 and R_2 being unidentified radicals. At the same time it was shown that these compounds were convertible by simple chemical methods, which have their counterpart in the animal body, to the final excretion products—8.OH benzpyrene and the 5:8 quinone. From the *in vitro* experiments with thiols Calcutt (1949) isolated a compound comparable to BpX_1 and in turn convertible, by the same methods used by Weigert and Mottram (1946*a*, 1946*b*), to a compound apparently identical with 8.OH benzpyrene. It is then with the formation of this BpX_1 type compound that the problem is concerned.

It was suggested by Quick (1937) that the primary step in the metabolic oxidation of hydrocarbons is a union of cysteine with the unsubstituted aromatic ring, replacement of the mercapturic acid by a hydroxyl group occurring later. There is no direct evidence for this view, but the recognized involvement between carcinogenesis and sulphur·metabolism (Crabtree, 1947; Calcutt, 1949) is in its favour. It is, however, severely criticized by Boyland and Weigert (1947), who point out that phenylcysteine is excreted as phenylmercapturic acid—evidence which suggests that the linkage between cysteine and an aromatic compound is not easily broken in the body.

Consideration of the metabolism of dibenzanthracene led Fieser (1941) to suggest the addition of hydrogen peroxide as the primary metabolic process. Loss of water would then give the phenolic derivative. A similar perhydroxylation mechanism has been suggested by Porteous and Williams (1949) as the

only feasible explanation of the isolated end-products of the metabolism of benzene. It is apparent that such a scheme could apply equally well in the case of the chemically related benzpyrene, BpX_1 or BpX_2 arising from the substitution of the hydrogen of one or other of the hydroxyl groups of the primarily formed dialcohol.

In the case of the *in vitro* experiments no evidence was found to indicate any linkage of the hydrocarbon and the thiol. Equally, even if such linkage had taken place it is difficult to conceive how the replacement by a hydroxyl group could have occurred under the simple conditions used. An alternative possibility in line with the perhydroxylation view is feasible since autoxidizing thiols are known to give rise to hydrogen peroxide (Schoberl, 1932; Holtz and Triem, 1937; Schales, 1938). Additionally, Weil-Malherbe (1947) claimed that benzpyrene was oxidized by hydrogen peroxide. Unfortunately no results are recorded as to the nature of the oxidized products.

If a perhydroxylation process is the initial step in both the *in vivo* and *in vitro* oxidation of benzpyrene it appears that it should be possible—under suitable conditions—to oxidize the hydrocarbon directly with hydrogen peroxide. This has been attempted, and the results are detailed below.

METHODS.

The oxidation of 3:4-benzpyrene with hydrogen peroxide.

The addition of 6 per cent hydrogen peroxide to a colloidal suspension of benzpyrene in distilled water had no effect at room temperature even when the two compounds were maintained together for long periods. If, however, a similar mixture was maintained at 55°C. for 30 minutes the typical yellow-green fluorescence of the benzpyrene was replaced by a blue fluorescence of the type associated with benzpyrene derivatives. Continued warming led to the apparent complete disappearance of the benzpyrene and the formation of a brownish, insoluble flocculent material. Since hydrogen peroxide contains acid as a stabilizing agent, any derivative of the BpX_1 type formed in the reaction would be expected to break down to the phenol and thence to the quinone—a sequence of events which superficially would resemble those found.

As an attempt to counteract any influence due to the acid the experiment was repeated using the benzpyrene colloid suspended in a Clark and Lubbs buffer solution at a pH of 7·5. This time the hydrogen peroxide was added slowly; whilst at intervals the pH was checked, and when necessary was restored to 7·5 by the further addition of 0·2M. NaoH solution. After 30 minutes the reaction had reached the blue fluorescent stage so the mixture was cooled in the attempt to stop further action.

RESULTS.

The isolation and examination of the reaction products.

The cooled reaction mixture was repeatedly extracted with purified (fluorescence free) xylene. The xylene was dried over anhydrous sodium sulphate and then passed through a chromatograph column packed with silica. After repeated washing with clean xylene there remained at the top of the column an extended zone which fluoresced pale blue under the U.V. lamp.

The column was extruded and the fluorescent zone was divided into three

portions. All three were then extracted with methyl alcohol. Examination of the fluorescent spectra of the solution derived from the three portions gave results as below :

Surface :—Two diffuse maxima at 420 mμ. and 440 mμ. Indistinguishable from fluorescence spectrum of BpX_1 derived from mice.

Middle :—Maxima as above but overshadowed by a diffuse background.

Bottom :—One extended diffuse zone without banded structure.

Further examination of the material from the surface zone showed it to be water-soluble with a pale blue fluorescence. The addition of a few drops of acid converted this material to an alkali-soluble product having a fluorescence spectrum identical with that of 8.OH benzpyrene. The likelihood of it actually being 8.OH benzpyrene is enhanced by the fact that after transfer to petroleum ether and standing for a few days it lost its blue fluorescence and achieved a barely perceptible yellowish tinge, behaviour which would be in keeping with the known autoxidation of 8.OH benzpyrene to benzpyrene 5:8 quinone. The solution was therefore passed through a silica column, which resulted in the formation of a faintly tinted zone. The passage of a weak solution of an authentic sample of 3:4-benzpyrene 5:8-quinone through the same column resulted in concentration over the previous zone, and, although the zone slowly passed down the column, no further elution or variation in solvent allowed any separation of the two zones. This must be regarded as confirmatory evidence that the derived material was benzpyrene 5:8 quinone. The similarity of fluorescence spectra, physical properties and behaviour suggests then that the material separated from the reaction mixture is similar to, even if not identical with, the BpX_1 from mice and the BpX_1-like material formed in the presence of autoxidizing thiols.

Further examination of the substances extracted from the other two portions of the original chromatograph column produced little of interest other than that the material was water-soluble in both cases. Acid had no determinable effect. The very limited amounts of substance available severely hindered any detailed examination, and thereby preclude any conclusions as to the nature of these compounds. If, however, a benzpyrene diol was formed, as appears likely from the evidence above, it is possible that these latter compounds are further oxidation products. In view of Boyland and Levi's (1935) finding that dihydroxydihydroanthracene is very susceptible to oxidation, such an occurrence would seem most probable.

Examination of the eluate from the original chromatograph column showed the presence of unchanged benzpyrene only.

Attempts to improve the reaction yield.

Despite repeated experiments it has so far proved impossible to obtain more than very small amounts of the reaction products. The reason for this appears to be that in the presence of the buffer the benzpyrene colloid undergoes coagulation, with a subsequent reduction in surface area. As soon as the benzpyrene becomes coated with the blue fluorescing material, which is insoluble in the alkaline buffer medium, the reaction ceases. Attempts to utilize the benzpyrene in solution in organic solvents have so far failed, the only results being the formation of small amounts of apparent 8.OH benzpyrene and quantities of various unidentifiable products. Such systems undoubtedly introduce the further problem of

the relative ease of oxidation of the solvent as compared with the benzpyrene. For the moment therefore the question of the isolation of sufficient of the BpX_1 type material for chemical identification is still unsolved.

DISCUSSION.

The work described above indicates that 3:4-benzpyrene can engage in direct action with hydrogen peroxide. Furthermore, the evidence suggests that the primary reaction product is a diol closely resembling that found as the initial product of benzpyrene metabolism. At the same time this primary product shows a remarkable resemblance to that found as the result of oxidation of benzpyrene in the presence of autoxidizing thiols. In view of the known formation of hydrogen peroxide during the autoxidation of –SH groups, it seems probable that the primary mechanism of oxidation in both cases consists of the addition of hydrogen peroxide to the hydrocarbon. Equally, since the formation of peroxide is a phenomenon known to occur during biological oxidations (Oppenheimer and Stern, 1939), it seems that such a mechanism could account for the initial steps of the *in vivo* oxidation of benzpyrene.

At the moment, however, no specific conclusions can be drawn, since the identity of the three similar substances cannot be proved. The problem must await the discovery of conditions which will allow of the isolation of the oxidation products in such quantity as to allow complete characterization.

SUMMARY.

The oxidation of 3:4-benzpyrene with hydrogen peroxide and the isolation of a product similar to the benzpyrene oxidation products obtained during metabolism and during oxidation in the presence of autoxidizing thiols is described. It is suggested that the oxidation of benzpyrene in the presence of autoxidizing thiols is due to hydrogen peroxide formation.

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